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TUESDAY—AFTERNOON SESSION

CONTRIBUTED PAPERS

Orrin Bowman Winter, 1878–1937

ORRIN BOWMAN WINTER

On the morning of October 1, 1937, there was ended in its 58th year the busy life of Orrin Bowman Winter. Death, from a heart attack, came suddenly and unexpectedly just before breakfast and shocked relatives and a host of friends alike. The Michigan Agricultural Experiment Station had lost one of its most faithful workers, and the Association of Official Agricultural Chemists one of its most valuable members.

Mr. Winter was born near Caledonia, Kent County, Michigan, on December 5, 1878, and received his early education in that locality. After two years at Michigan State Normal College and several years of high school teaching he entered the University of Michigan to begin his work in chemistry. There, in class and as an assistant, he came under the magic spell of Professor Gomberg and received an inspiration which, through all the subsequent years, he never permitted to lag. In 1909 he graduated with the degree of Bachelor of Science, and immediately there began an association with the Michigan Experiment Station which, with the exception of two years (1911 and 1912) at the New York Geneva Station with Dr. L. L. Van Slyke, was not broken until his death 28 years later.

An adequate review of the diversified subjects that have been studied and published by Mr. Winter is quite without the province of this communication. Some idea, however, may be obtained from the following excerpts from a few titles of his journal articles and station bulletins: The Estimation of Calcium and Strontium in the Presence of Phosphoric Acid and Iron; A Contribution to the Composition of Lime Sulfur Solutions; The Electrometric Titration of Arsenicals; Studies of the Availability of Organic Nitrogenous Compounds; The Microscopic Identification and Determination of the Specific Ingredients in Stock Feeds; Determination of Iron and Aluminum in the Presence of Calcium, Magnesium and Phosphoric Acid; The Determination of Aluminum in Plants; Fermentation Studies with Soft Wheat Flours; Volumetric Method for Determination of Fluorine; and A Modification of the Fischer-Leopoldi Method for the Determination of Lead.

Many of the methods of analysis published were devised by Winter through necessity, because existing methods were too slow or insufficiently sensitive to cope with the needs of an urgent problem, as for example the determination of lead in a large number of spray residues. Other methods were the results of years of study and experience, as was the case with those for the microscopic identification of specific ingredients of stockfeeds, which were developed during the years when the feed, fertilizer, and insecticide control work was done by the Station. Still others were developed in response to the recognition of a wide-spread need for a suitable method. Such a one was the Willard-Winter volumetric method for the determination of fluorine, the development of which may be likened to the removal of the key log in a jam and which has been followed by a veritable flood of modifications from numerous laboratories.

During the years in which he served the Michigan Station as Assistant, Research Assistant, and Research Associate in the Chemical Section, he not only conducted many research projects, but he did an amazing amount of routine work—in the earlier years in connection

with the feed, fertilizer, and insecticide control work, and in later years in connection with cooperative projects with other Sections of the Experiment Station. Throughout the years he also found time to keep abreast of current developments, and as late as 1930 returned to his Alma Mater to take graduate work and receive the Master of Science degree.

Mr. Winter's affiliation with the Association of Official Agricultural Chemists began many years ago, when he was associated with Professor A. J. Patten and Dr. C. S. Robinson on numerous collaborative studies on methods. Since 1928 he had been Referee on Plants, and his annual reports published in the *Journal* attest the faithful performance of his duties in that office.

Among the outstanding characteristics of the man were his faithful devotion and loyalty to his family, his friends, and his work. He was a tireless worker. Laboratory processes were frequently kept going day and night, and made necessary long hours and frequent trips to the laboratory evenings, Saturday afternoons, and holidays. This routine was possible partly because during all the years he was connected with the Station his home was within a few minutes' walk of the laboratory. As a result he accomplished more in the relatively short span of life allotted to him than did many another in three score and ten years. Perhaps an unexpressed feeling that his time might be short because of a heart involvement that followed pneumonia when he was 22 years of age impelled him to make the best of every day.

All who knew Mr. Winter will remember him for his cheerful, pleasing personality, his unselfishness, and his willingness to help at whatever cost to himself. He was a member of the Peoples Church of East Lansing for a quarter of a century and for years he was a member of the Board of Elders. For the past seven years he had been Superintendent of the Junior Department of the Church School, and for a long period previously had taught in the same department. This interest was shared by Mrs. Winter, who has likewise been Superintendent of the Primary Department.

Mr. Winter also served his associates in his chosen profession. He was a member of the American Chemical Society, being a charter member of the Michigan State College Section and serving as Chairman in 1933 and as Treasurer for years before that. He was a member of the American Association of Cereal Chemists and of the honorary and professional societies, Sigma Xi, Phi Sigma, and Phi Lambda Upsilon.

Mr. Winter is survived by his widow, Ethel Gilpin Winter, by two sons, Gerald Gilpin and Richard Gilpin Winter, and by a legion of friends and associates in his church, the Michigan Experiment Station, and the A.O.A.C., all of whom will agree that the words, "Well done thou good and faithful servant," were never more fittingly applicable than they are to Orrin Bowman Winter.

E. J. Miller

PROCEEDINGS OF THE FIFTY-THIRD ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1937

The fifty-third annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., November 1, 2, and 3, 1937.

The meeting was called to order by the president, C. C. McDonnell, U. S. Food and Drug Administration, Washington, D. C., on the morning of November 1, at 10:40 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING NOVEMBER, 1938

President

H. R. Kraybill, Purdue University, Lafayette, Ind.

Vice-President

W. S. Frisbie, U. S. Food and Drug Administration, Washington, D. C.

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W. S. Sale, Washington, D. C.
G. G. Frary, Vermillion, S.D.
C. C. McDonnell, Washington, D. C.

Permanent Committees

Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires.)

H. A. Lepper (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*

Subcommittee A.: G. E. Grattan (1940), (Department of Agriculture, Ottawa, Can.), *Chairman;* H. A. Halvorson (1942) and E. L. Griffin (1938). [Standard solutions; insecticides, fungicides, and caustic poisons (fluorine compounds, pyrethrins, derris, and cube, naphthalene in poultry lice products); soils and liming materials (hydrogen-ion concentration—soils of arid and semi-arid regions and soils of humid regions, liming materials, less common metals in soils, selenium); feeding stuffs (sampling, ash, mineral mixed feeds—calcium and iodine, moisture, lactose in mixed feeds, fat in fish meal, hydrocyanic acid in glucoside-bearing materials, biological methods for determination of vitamin D carriers, biological methods for vitamin B complexes, technic and details of biological methods, vitamin D carriers, carotene, qualitative tests for proteins, manganese, adulteration of condensed milk products and of cod liver oil); fertilizers (phosphoric acid, nitrogen, magnesium and manganese, acid and base-forming quality, potash, calcium, sulfur, copper, zinc); plants (less com-

mon metals, total chlorine, carbohydrates, inulin, hydrocyanic acid, forms of nitrogen, sodium and potassium); lignin, enzymes (papain), paints, paint materials and varnishes (accelerated testing of paints, varnishes); vitamins (vitamin A, vitamin D); leathers and tanning materials, disinfectants.]

SUBCOMMITTEE B: L. B. Broughton (1938), (University of Maryland, College Park, Md.), *Chairman;* H. J. Fisher (1940) and A. E. Paul (1942). [Naval stores (rosin, turpentine); radioactivity (quantum counter, gamma ray scope), cosmetics, drugs (microchemical methods for alkaloids, microchemical methods for synthetics, hypophosphites, daphnia methods, hexylresorcinol, ergot alkaloids, nitroglycerin in mixtures, guaiacol, biological testing, iodine ointment, acetophenetidin in presence of caffeine and aspirin, gums, theobromine-calcium tablets, chlorbutanol, aspirin and phenolphthalein mixtures, emulsions, elixir of terpin hydrate and codeine, aminopyrine and phenobarbital in mixtures, ointment of mercuric nitrate, rhubarb and rhaponticum, theophylline, sulfanilamide, mandelic acid.]

SUBCOMMITTEE C: J. O. Clarke (1938), (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman;* G. G. Frary (1940) and W. B. White (1942). [Dairy products (butter—preparation of sample and fat, cheese, malted milk (chemical methods, microanalytical methods), dried milk, milk proteins, lactose in milk, gelatin in milk and cream, citric acid in milk, extraneous matter, decomposition, neutralizers, tests for pasteurization of dairy products, difference between dairy products made from cow's milk and those made from milk of other animals); oils, fats, and waxes (refractometric determination of oil in seeds, thiocyanogen number, Polenski number); eggs and egg products (unsaponifiable constituents and fat, detection of decomposition, glycerol, sugar, and added salt, dried eggs); metals in foods (arsenic and antimony, copper, zinc, fluorine, lead, mercury, selenium, fumigation residues); canned foods (tomato products), vinegars (ash), meats and meat products, gums in foods, spices, microbiological methods—canned foods (canned fish products, canned meats, canned vegetables, canned tomato products, eggs and egg products, sugar); fish and other marine products.]

SUBCOMMITTEE D: J. A. LeClerc (1940), (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Chairman;* W. C. Jones (1940) and J. W. Sale (1942). Sugars and sugar products (acetyl-methyl carbinol and diacetyl in food products, unfermentable sugars in molasses, honey, refractive indices of sugar solutions, maple products; drying, densimetric, and refractometric methods; polariscopic methods, chemical methods for reducing sugars); waters and effervescent salts) alcoholic beverages (diastatic activity of malt, proteolytic activity of malt, malt extract in malt, malt adjuncts, beer, CO_2 in beer, heavy metals in beer, total sulfur and lead esterification, volatile acids in wines, volatile acids in distilled spirits, SO_2 in wines and beers, aldehydes in whiskey and other potable spirits, potentiometric titration distilled spirits, cordials and liqueurs); food preservatives (saccharin, benzoate of soda); coloring matters in foods, fruits and fruit products (soluble solids and effect of acids on sugar on drying, electrometric titration of acids, malic, isocitric, and lactic acids, P_2O_5 in jams, jellies, and other fruit products, polariscopic methods for jams, jellies, and preserves); flavors and non-alcoholic beverages, cacao products, cereal foods (soya flour in foods, macaroni, whole wheat flour, phosphated flour; ash in flour, macaroni products, and baked products; H-ion concentration of flour, acidity of flour, sugar in flour, starch in flour, flour-bleaching chemicals, CO_2 in self-rising flour, milk solids in milk bread, viscosity of flour, cold water extract

flour, ergot in flour, proteolytic enzymes, color in flour, baking test for soft wheat flour); microchemical methods, flavors and non-alcoholic beverages (organic solvents in flavors), cacao products.]

Committee to Cooperate with Other Committees on Food Definitions

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E. M. Bailey — G. G. Frary

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L. E. Warren — G. G. Frary

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Representatives on the Board of Governors of the Crop Protection Institute of the National Research Council

H. J. Patterson, College Park, Md.

W. H. MacIntire, Knoxville, Tenn.

Referees and Associate Referees

Standard solutions:

General referee: R. L. Vandaveer, Food and Drug Adm., Chicago, Ill.

INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS:

General referee: J. J. T. Graham, Food and Drug Adm., Washington, D. C.

PYRETHRINS, DERRIS, AND CUBE:

Associate referee: J. J. T. Graham.

FLUORINE COMPOUNDS:

Associate referee: R. H. Carter, Bureau of Entomology and Plant Quarantine, Washington, D. C.

NAPHTHALENE IN POULTRY LICE PRODUCTS:

Associate referee: Roswell Jinkins, Food and Drug Adm., Chicago, Ill.

SOILS AND LIMING MATERIALS:

General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

HYDROGEN-ION CONCENTRATION:

a. SOILS OF ARID AND SEMI-ARID REGIONS:

Associate referee: W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

b. SOILS OF HUMID REGIONS:

Associate referee: E. R. Purvis, Virginia Truck Experiment Station, Norfolk, Va.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

SELENIUM IN SOILS:

Associate referee: K. T. Williams, Bureau of Chemistry and Soils, Washington, D. C.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

FEEDING STUFFS:

General referee: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

SAMPLING:

Associate referee: L. M. Jeffers, Dept. of Agriculture, Sacramento, Calif.

ASH:

Associate referee: J. L. St. John, Agricultural Experiment Station, Pullman, Wash.

MINERAL MIXED FEEDS (calcium and iodine):

Associate referee: H. E. Perkins, Manhattan, Kans.

LACTOSE IN MIXED FEEDS:

Associate referee: D. A. Magraw, American Dry Milk Inst., Chicago, Ill.

MOISTURE:

Committee: H. A. Halvorson, P. B. Curtis, and P. A. Clifford.

Hydrocyanic acid in glucoside-bearing materials:

Associate referee: R. A. Greene, University of Arizona, Tucson, Ariz.

Biological methods for determination of vitamin D carriers:

Associate referee: W. B. Griem, Department of Agriculture and Markets, Madison, Wis.

Biological methods for vitamin B complexes:

Associate referee: O. L. Kline, Food and Drug Administration, Washington, D. C.

Technic and details of biological methods, vitamin D carriers:

Associate referee:

Manganese:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

Carotene:

Associate referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

Qualitative tests for proteins:

Associate referee: D. Breese Jones, Bureau of Chemistry and Soils, Washington, D. C.

Fat in fish meal:

Associate referee: R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

Adulteration of condensed milk products and cod-liver oil:

Associate referee: P. B. Curtis, Agricultural Experiment Station, Lafayette, Ind.

Fertilizers:

General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

Phosphoric acid:

Associate referee: W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

Nitrogen:

Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

Magnesium and manganese:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

Potash:

Associate referee: O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

Acid and base-forming quality:

Associate referee: L. E. Horat, Agricultural Experiment Station, Lafayette, Ind.

CALCIUM, SULFUR, COPPER, ZINC:

Associate referee: Gordon Hart, Department of Agriculture, Tallahassee, Fla.

PLANTS:

General referee: E. J. Miller, Agricultural Experiment Station, E. Lansing, Mich.

LESS COMMON METALS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE:

Associate referee: H. L. Wilkins, Bureau of Plant Industry, Washington, D. C.

CARBOHYDRATES:

Associate referee: J. T. Sullivan, Agricultural Experiment Station, Lafayette, Ind.

INULIN:

Associate referee: T. G. Phillips, University of New Hamphsire, Durham, N. H.

FORMS OF NITROGEN:

Associate referee: H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

HYDROCYANIC ACID:

Associate referee: R. A. Greene, University of Arizona, Tucson, Ariz.

SODIUM AND POTASSIUM:

Associate referee. R. T. Milner, Regional Soybean Industrial Products Lab., Urbana, Ill.

LIGNIN:

General referee: M. Phillips, Bureau of Chemistry and Soils, Washington, D. C.

ENZYMES:

General referee: A. K. Balls, Bureau of Chemistry and Soils, Washington, D. C.

PAPAIN:

Associate referee: T. L. Swenson, Bureau of Chemistry and Soils, Washington, D. C.

PAINTS, PAINT MATERIALS AND VARNISHES:

General referee: C. S. Ladd, Food Commissioner and Chemist, Bismarck, N. D.

ACCELERATING TESTING OF PAINTS:

Associate referee: L. L. Carrick, Agricultural Experiment Station, Fargo, N. D.

VARNISHES:

Associate referee: F. Roberts, Paint and Varnish Lab., Bismarck, N. D.

VITAMINS:

General referee: E. M. Nelson, Food and Drug Adm., Washington, D. C.

VITAMIN A:

Associate referee: J. B. Wilkie, Food and Drug Adm., Washington, D. C.

VITAMIN D:

Associate referee: W. C. Russell, Agricultural Experiment Station, New Brunswick, N. J.

LEATHERS AND TANNING MATERIALS:

General referee: I. D. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

DISINFECTANTS:

General referee: C. N. Brewer, Food and Drug Adm., Washington, D. C.

NAVAL STORES:

General referee: F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

ROSIN:

Associate referee: F. P. Veitch.

TURPENTINE:

Associate referee: V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

RADIOACTIVITY:

General referee: C. H. Badger, Food and Drug Adm., Washington, D. C.

QUANTUM COUNTER:

Associate referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

GAMMA RAY SCOPE:

Associate referee: C. H. Badger.

COSMETICS:

General referee: E. W. Campbell, Bureau of Health, Augusta, Me.

DRUGS:

General referee: L. E. Warren, Food and Drug Adm., Washington, D. C.

ACETOPHENETIDIN IN PRESENCE OF CAFFEINE AND ASPIRIN:

Associate referee: D. C. Grove, Food and Drug Adm., Washington, D. C.

AMINOPYRINE AND PHENOBARBITAL IN MIXTURES:

Associate referee: E. C. Payne, Food and Drug Adm., Chicago, Ill.

ASPIRIN AND PHENOLPHTHALEIN MIXTURES:

Associate referee: G. M. Johnson, Food and Drug Adm., Minneapolis, Minn.

BIOLOGICAL TESTING:

Associate referee: J. C. Krantz, Jr., University of Maryland, College Park, Md.

CHLORBUTANOL:

Associate referee: F. C. Sinton, Food and Drug Adm., New York City.

DAPHNIA METHODS:

Associate referee: A. Viehoever, College of Pharmacy and Science, Philadelphia, Pa.

ELIXIR OF TERPIN HYDRATE AND CODEINE:

Associate referee: Jonas Carol, Food and Drug Adm., Cincinnati, O.

EMULSIONS:

Associate referee: W. F. Kunke, Food and Drug Adm., Chicago, Ill.

ERGOT ALKALOIDS:

Associate referee: Lloyd C. Miller, Food and Drug Adm., Washington, D. C.

GUAIACOL:

Associate referee: K. L. Milstead, Food and Drug Adm., Chicago, Ill.

GUMS:

Associate referee: J. H. Cannon, Food and Drug Adm., Chicago, Ill.

HEXYLRESORCINOL:

Associate referee: M. L. Yakovitz, Food and Drug Adm., San Francisco, Calif.

SULFANILAMIDE:

Associate referee: E. M. Hoshall, Food and Drug Adm., Baltimore, Md.

HYPOPHOSPHITES:

Associate referee: H. R. Bond, Food and Drug Adm., Chicago, Ill.

IODINE OINTMENT:

Associate referee: W. F. Reindollar, State Dept. of Health, Baltimore, Md.

MICROCHEMICAL METHODS FOR ALKALOIDS:

Associate referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR SYNTHETICS:

Associate referee: I. S. Shupe, Food and Drug Adm., St. Louis, Mo.

NITROGLYCERINE IN MIXTURES:

Associate referee: O. A. Kenworthy, Food and Drug Adm., New York City.

OINTMENT OF MERCURIC NITRATE:

Associate referee: H. O. Moraw, Food and Drug Adm., Chicago, Ill.

THEOPHYLLINE SODIUM SALICYLATE:

Associate referee: M. Harris, Food and Drug Adm., Chicago, Ill.

MANDELIC ACID:

Associate referee: H. G. Underwood, Food and Drug Adm., Cincinnati, Ohio.

Rhubarb and rhaponticum:

Associate referee: E. H. Wirth, University of Illinois School of Pharmacy, Chicago, Ill.

Theobromine and Theobromine-calcium tablets:

Associate referee: P. S. Jorgensen, Food and Drug Adm., San Francisco, Calif.

Dairy products:

General referee: G. G. Frary, Dairy and Food Dept., Vermillion, S. D.

Butter—preparation of sample and fat:

Associate referee: R. S. Pruitt, Food and Drug Adm., New Orleans, La.

Cheese:

Associate referee: I. D. Garard, Rutgers University, New Brunswick, N. J.

Malted milk, chemical methods:

Associate referee: F. Hillig, Food and Drug Adm., Washington, D. C.

Malted milk, microanalytical methods:

Associate referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

Dried milk:

Associate referee: F. Hillig.

Milk proteins:

Associate referee: W. E. Peterson, College of Agriculture, St. Paul, Minn.

Gelatin in milk and cream:

Associate referee: G. A. Richardson, University of California, Davis, Calif.

Lactose in milk:

Associate referee: E. R. Garrison, University of Missouri, Columbia, Mo.

Extraneous matter in dairy products:

Associate referee: J. D. Wildman, Food and Drug Adm., Washington, D. C.

Citric acid in milk:

Associate referee: B. G. Hartmann, Food and Drug Adm. Washington, D. C.

Tests for pasteurization of dairy products:

Associate referee: F. W. Gilcreas, Department of Health, Albany, N. Y.

Difference between dairy products made from cow's milk and those made from the milk of other animals:

Associate referee: I. D. Garard.

Decomposition in dairy products:

Associate referee: C. S. Myers, Food and Drug Adm., Washington, D. C.

Neutralizers in dairy products:

Associate referee: F. Hillig,

OILS, FATS AND WAXES:

General referee: G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:

Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

THIOCYANOGEN NUMBER:

Associate referee: G. S. Jamieson.

POLENSKI NUMBER:

Associate referee: R. S. McKinney, Bureau of Chemistry and Soils, Washington, D. C.

EGGS AND EGG PRODUCTS:

General referee: H. A. Lepper, Food and Drug Adm., Washington, D. C.

UNSAPONIFIABLE CONSTITUTENTS AND FAT:

Associate referee: E. O. Haenni, Food and Drug Adm., Washington, D. C.

GLYCEROL, SUGAR AND ADDED SALT:

Associate referee: L. C. Mitchell, Food and Drug Adm., Minneapolis, Minn.

DETECTION OF DECOMPOSITION:

Associate referee: J. Callaway, Jr., Food and Drug Adm., New York City.

DRIED EGGS:

Associate referee: F. J. McNall, Food and Drug Adm., Chicago, Ill.

METALS IN FOODS:

General referee: H. J. Wichmann, Food and Drug Adm., Washington, D. C.

ARSENIC AND ANTIMONY:

Associate referee: C. C. Cassil, Bureau of Entomology and Plant Quarantine, Washington, D. C.

COPPER:

Associate referee: D. L. Drabkin, University of Pennsylvania, Philadelphia, Pa.

ZINC:

Associate referee: W. S. Ritchie, Agricultural Experiment Station, Amherst, Mass.

FLUORINE:

Associate referee: Dan Dahle, Food and Drug Adm., Washington, D. C.

LEAD:

Associate referee: P. A. Clifford, Food and Drug Adm., Washington, D. C.

MERCURY:

Associate referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

SELENIUM:

Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

FUMIGATION RESIDUES IN FOODS:

Associate referee: W. O. Winkler.

COLORING MATTERS IN FOODS:

General referee: C. F. Jablonski, Food and Drug Adm., New York City.

FRUITS AND FRUIT PRODUCTS:

General referee: B. G. Hartmann, Food and Drug Adm. Washington, D. C.

SOLUBLE SOLIDS AND EFFECT OF ACIDS ON SUGAR ON DRYING:

Associate referee: C. H. Badger, Food and Drug Adm., Washington, D. C.

ELECTROMETRIC TITRATION OF ACIDS:

Associate referee: R. U. Bonnar, Food and Drug Adm., Washington, D. C.

MALIC, ISOCITRIC, AND LACTIC ACIDS:

Associate referee: B. G. Hartmann.

P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS:

Associate referee: H. Shuman, Food and Drug Adm., Philadelphia, Pa.

POLARISCOPIC METHODS FOR JAMS, JELLIES, AND PRESERVES:

Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

CANNED FOODS:

General referee: V. B. Bonney, Food and Drug Adm., Washington, D. C.

TOMATO PRODUCTS:

Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

VINEGARS:

General referee: A. M. Henry, Food and Drug Adm., Atlanta, Ga.

ASH:

Associate referee: H. Shuman, Food and Drug Adm., Philadelphia, Pa.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

General referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

ORGANIC SOLVENTS IN FLAVORS:

Associate referee: R. D. Stanley, Food and Drug Adm., Chicago, Ill.

MEAT AND MEAT PRODUCTS:

General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:

General referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

GUMS IN FOODS:

General referee: F. Leslie Hart, Food and Drug Adm., Los Angeles, Calif.

SPICES:

General referee: J. F. Clevenger, Food and Drug Adm., New York City.

MICROBIOLOGICAL METHODS:

General referee: A. C. Hunter, Food and Drug Adm., Washington, D. C.

CANNED FISH PRODUCTS:

Associate referee: O. W. Lang, Hooper Foundation Medical Research, University of California, San Francisco, Calif.

CANNED MEATS:

Associate referee: L. B. Jensen, Swift & Co., Chicago, Ill.

CANNED VEGETABLES:

Associate referee: E. J. Cameron, National Canners Assn., Washington, D. C.

CANNED TOMATO PRODUCTS:

Associate referee: B. A. Linden, Food and Drug Adm., Washington, D. C.

SUGAR:

Associate referee: E. J. Cameron.

EGGS AND EGG PRODUCTS:

Associate referee: Roy Schneiter, Food and Drug Adm., Washington, D. C.

FISH AND OTHER MARINE PRODUCTS:

General referee: H. D. Grigsby, Food and Drug Adm., Philadelphia, Pa.

SUGARS AND SUGAR PRODUCTS:

General referee: R. F. Jackson, National Bureau of Standards, Washington, D. C.

ACETYL-METHYL CARBINOL AND DIACETYL IN FOOD PRODUCTS:

Associate referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

UNFERMENTABLE SUGARS IN MOLASSES:

Associate referee: Harry E. Goresline, Bureau of Chemistry and Soils, Washington, D. C.

HONEY:

Associate referee: R. E. Lothrop, Bureau of Chemistry and Soils, Washington, D. C.

MAPLE PRODUCTS:

Associate referee: J. F. Snell, Macdonald College, Quebec, Canada.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:

Associate referee: C. F. Snyder, National Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS(GENERAL):

Associate referee: Ralph M. Kingsbury, Bureau of Chemistry and Soils, Washington, D. C.

CHEMICAL METHODS FOR REDUCING SUGARS:

Associate referee: R. F. Jackson.

REFRACTIVE INDICES OF SUGAR SOLUTIONS:

Associate referee: R. T. Balch, Bureau of Chemistry and Soils, Washington, D. C.

WATERS, AND EFFERVESCENT SALTS:

General referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

CEREAL FOODS:

General referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS:

Associate referee: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

H-ION CONCENTRATION OF FLOUR:

Associate referee: George Garnatz, The Kroger Food Foundation, Cincinnati, Ohio.

ACIDITY IN FLOUR:

Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

STARCH IN FLOUR:

Associate referee. C. Y. Hopkins, National Research Council, Ottawa, Canada.

SUGAR IN FLOUR:

Associate referee: R. M. Sanstedt, Agricultural Experiment Station, Lincoln, Nebr.

BAKING TEST FOR SOFT WHEAT FLOUR:

Associate referee: E. G. Bayfield, Agricultural Experiment Station, Wooster, Ohio.

FLOUR-BLEACHING CHEMICALS:

Associate referee: Dorothy Scott, Food and Drug Adm., New York City.

CO_2 IN SELF-RISING FLOUR:

Associate referee: Rufus A. Barackman, Victor Chem. Works, Chicago Heights, Ill.

MILK SOLIDS IN MILK BREAD:

Associate referee: V. E. Munsey.

COLD WATER EXTRACT FLOUR:

Associate referee: H. C. Fellows, Bureau of Agricultural Economics, Washington, D. C.

ERGOT IN FLOUR:

Associate referee: Lloyd C. Miller, Food and Drug Adm., Washington, D. C.

PROTEOLYTIC ENZYMES:

Associate referee: Quick Landis, Fleischmann Laboratories, New York City.

COLOR IN FLOUR:

Associate referee: H. K. Parker, Novadel-Agene Corporation, Newark, N. J.

SOYA FLOUR IN FOODS:

Associate referee: J. W. Hayward, Archer-Daniels-Midland Co., Milwaukee, Wisc.

MACARONI:

Associate referee: W. F. Geddes, Board of Grain Commissioners, Winnipeg, Can.

WHOLE WHEAT FLOUR:

Associate referee: C. S. Ladd, N. Dakota Regulatory Dept., Bismarck, N. Dak.

PHOSPHATED FLOUR:

Associate referee: J. R. Davies, Calumet Baking Powder Co., Chicago, Ill.

BAKING POWDERS—TARTRATES:

General referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

MICROCHEMICAL METHODS:

Associate referee: E. P. Clark, Bureau of Entomology and Plant Quarantine, Washington, D. C.

ALCOHOLIC BEVERAGES:

General referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

DIASTATIC ACTIVITY OF MALT:

Associate referee: Christian Rask, Albert Schwill Co., Chicago, Ill.

PROTEOLYTIC ACTIVITY OF MALT:

Associate referee: Stephen Laufer, Schwartz Laboratories, Inc., New York City.

HEAVY METALS IN BEER:

Associate referee: W. H. Harrison, Continental Can Co., Chicago, Ill.

CARBON DIOXIDE IN BEER:

Associate referee: P. P. Gray, Wallerstein Laboratories, New York City.

MALT EXTRACT IN MALT:

Associate referee: E. A. Siebel, 8 S. Dearborn St., Chicago, Ill.

MALT ADJUNCTS:

Associate referee: F. P. Siebel, Siebel Institute, Chicago, Ill.

BEER:

Associate referee: H. W. Rohde, Schlitz Brewing Co., Milwaukee, Wisc.

TOTAL SULFUR AND LEAD ESTERIFICATION:

Associate referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

VOLATILE ACIDS IN WINES:

Associate referee: M. A. Joslyn, Agricultural Experiment Station, Berkeley, Calif.

SULFUR DIOXIDE IN BEERS AND WINES:

Associate referee: L. V. Taylor, American Can Co., Maywood, Ill.

VOLATILE ACIDS IN DISTILLED SPIRITS:

Associate referee: G. F. Beyer, Bureau of Internal Revenue, Washington, D. C.

ALDEHYDES IN WHISKEY AND OTHER POTABLE SPIRITS:

Associate referee: Peter Valaer, Bureau of Internal Revenue, Washington, D. C.

DETECTION OF ADULTERATION OF DISTILLED SPIRITS:

Associate referee: S. T. Schicktanz, Bureau of Internal Revenue, Washington, D. C.

WOOD ALCOHOL IN BRANDY:

Associate referee: G. F. Beyer.

CORDIALS AND LIQUEURS:

Associate referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

FOOD PRESERVATIVES—SACCHARIN:

General referee: W. F. Reindollar, Bureau of Chemistry, Baltimore, Md.

BENZOATE OF SODA:

Associate referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

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WILEY MEMORIAL LECTURE. No. VII

UNSOLVED PROBLEMS OF AGRICULTURAL CHEMISTRY*

By C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.)

It is most fitting that we should begin these annual conventions by commemorating the services of the man who was a leader in the movement that led to the foundation of our Association and who for nearly half a century, as secretary and honorary president, was the guiding spirit of its varied activities. Although nine years have passed since Dr. Wiley's voice was last heard in this hall, the recollection of his presence is still vivid among those of us who knew him. This memory is more than a tradition; it is a permanent influence, destined to be felt as long as there shall be in America men who are interested in the applications of chemistry to agriculture and to the public welfare.

We measure progress in spans of human life and only a few of these connecting links take us back to the dawn of our science. When Dr. Wiley was born in 1844, Berzelius, the master analyst and founder of our system of chemical notation, was still living. When Berzelius was born in 1779, Duhamel, the noted French agricultural chemist and author, was still active. When Duhamel was born in 1700, Grew, the famous London microscopist and chemist of plant life, had twelve more years of productive work before him. When Grew was born in 1641, van Helmont, the great pioneer of experimental agricultural chemistry, was still busy in his Belgian laboratory. When Helmont was born in 1577, Palissy, the Huguenot agricultural and industrial chemist, had twelve more years of active public service to render before he died a victim of religious persecution in the dungeons of the Bastile. Palissy was born in 1499, and thus it is seen that the spans of only six human lives carry us back from the twentieth to the fifteenth century, when chemistry had not yet emerged as a science.

This chemical genealogy, so briefly sketched, is a notable one, and if time permitted we might trace some interesting parallels between the lives of the men just mentioned and the career of Dr. Wiley. All the chemists named were versatile men of broad vision, interested in the applications of chemistry to the problems of plant and animal life, and for the most part ardently devoted, as was Dr. Wiley, to the public welfare. Each contributed his small share towards erecting the splendid edifice of agricultural chemistry as it exists today—an edifice which, although imposing, is still far from complete. It is about a few unfinished parts of this structure that I wish to address you this morning.

THE TERM "AGRICULTURAL CHEMISTRY"

Agricultural chemistry, the same as medical chemistry, industrial chemistry, sanitary chemistry, and other similar designations, is one of

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those borderland fields of knowledge where chemistry is applied to some special useful end. The propriety of designating as a separate science any branch of learning, where chemistry, physics, biology, geology, meteorology, and plant and animal physiology are all so mutually involved, as they are in agriculture, has long been questioned. In so far as chemistry is applied to the art of agriculture it is a part of the general science of chemistry and logically there would seem to be no more reason for setting up a science of agricultural chemistry than there would be for having one of agricultural physics or of agricultural biology. For this reason some of the classic writers, as Boussingault and Johnson, avoided so far as possible the use of the designation agricultural chemistry. We see even now that some leading agricultural colleges are dropping this term from their list of courses and that some well-recognized agricultural chemists are hiding their identities under such titles as agronomists, biochemists, and agrobiologists. The term agricultural chemistry, however, is not one of which we need feel ashamed, for it has the sanction of many great authorities, such as Davy, Deherain, and Adolf Mayer. Hermbstaedt was one of the first to employ the term in 1804 as a convenient title of his "Archiv der Agriculturchemie," a publication of wide scope in which the various contributors discussed not only problems of chemistry but those of physics, geology, meteorology, plant physiology, animal nutrition, agricultural technology, and national economy. We thus see at the very beginning, how seeds of confusion were sown about the meaning of the term agricultural chemistry—seeds that in later times were to produce abundant crops of misunderstanding.

There is, however, a need for retaining the term agricultural chemistry, if only as a matter of convenience, and no difficulty need arise from its use if we are careful to delimit the field and keep within its boundaries. I would define agricultural chemistry as "the chemistry of farm operations whether performed by nature or by man," or more specifically as, "that branch of chemistry which treats of the chemical composition and mutual chemical relationships of soils, crops, and farm animals so far as they concern the production of the means of human subsistence and comfort." The various transformations of the raw materials of the farm produced by the natural processes of weathering, microbial action, growth, decay, plant and animal metabolism, and the like, and the conversion upon the farm of these raw materials into butter, sugar, wine, starch, vegetable oils, food stuffs, and other rural commodities come within the recognized purview of agricultural chemistry. The utilization of agricultural produce beyond the frontiers of the farm, or cooperative, falls outside the scope of our definition.

ADVANCEMENTS DUE TO CHEMICAL ANALYSIS

As a society of analysts we may take just pride in the observation that the great advancements made in agricultural chemistry since the

beginning of the last century are wholly the result of improvements in the art of chemical analysis. The doctrine of special elementary transformations by means of plant life, which for over two centuries had obscured the vision of such chemists as van Helmont and Boyle, was definitely disproved by the analytical skill of Theodore de Saussure, whose "Recherches chimiques sur la Vegetation," published in 1804, placed crop chemistry for the first time upon a secure basis. Again thirty years later it was the patient work of Fresenius in systematizing and improving the methods of qualitative and quantitative analysis that helped the new doctrines of his teacher Liebig to win so rapid a victory. At the end of the century the introduction of improved analytical methods in organic, physical and biological chemistry greatly widened our knowledge of the composition and mutual chemical relationships of soils, crops, and animals. And so again today agricultural chemistry is passing through another period of progressive transition when the application of analytical and biological methods of higher refinement to the examination of plant and animal products is enabling us to identify and study the action of minute traces of hitherto neglected mineral elements and of those almost intangible entities, most potent for good or harm, that are variously designated as vitamins, enzymes, auxines, viruses, and hormones. It has seemed at times to agricultural chemists as if they had come to the very frontiers of their science, yet the attainment of another pinnacle revealed to their gaze a vast sea of unsolved problems beyond, and, like the followers of Cortez who were overcome with wonder at their first view of the Pacific, they

> Looked at each other with a wild surmise
> Silent upon a peak in Darien.

Consider one of the smallest, yet one of the most important, objects in all agriculture, that of a germinating seed and observe how much remains to be known about it. Of the chemical constituents of the germ, endosperm, aleurone layer, and epidermis, we have acquired a fairly general but by no means complete knowledge; of the mutual chemical relationships of these constituents we know much less; while of the exact nature of the complex chemical reactions that are involved in the process of germination we know very little. The various steps employed by the developing germ in utilizing the stores of food so carefully segregated by the plant for its need, and, when these stores are exhausted, the processes by which the young seedling elaborates from the soil and atmosphere the materials required for its independent existence, are only imperfectly understood. The seedlings of each species of crop and, what is less generally recognized, of each variety of crop differ greatly in their nutritive requirements and selective powers. One variety of sugar cane thrives luxuriantly in a soil in which another variety succumbs from lack of

nourishment. We can still put the question that we asked ourselves in that nursery rhyme of early childhood—

> Do you or I or anyone know
> How oats, peas, beans and barley grow?

A complete chemical history of a single crop has not yet been written and, we are safe in saying, never will be written. As remarked by me in an address that was given before the American Oil Chemists Society in 1925,—

> Such a history involves a knowledge of every chemical compound in the roots, stalks, leaves, fruits and other organs of the plant; of the manner in which these various compounds are produced; of the chemical processes which underlie all the various physiological activities such as germination, assimilation and growth; of the chemical reactions involved in the relations between the plant and its surrounding, such as soil, water, air, sunlight, insects, bacteria and other externals; and finally of the chemical utilization of the various parts of the plant for the specific needs of commerce and industry. Even the crops which have been most studied, such as corn or wheat, appear to the chemist only in the resemblance of an unsolved, very complicated crossword puzzle; with here and there a section filled in, with a few suggestive key words perhaps inserted, but with many blank spaces still remaining to be worked out.[1]

The stupendous magnitude of the undertaking, however, should not deter us from doing our part. The conquest of new unexplored fields in agricultural chemistry is wholly dependent upon the discovery of new analytical methods of higher refinement, and the work of this Association in the perfection of such methods will be of the greatest assistance in extending the frontiers of our knowledge.

In a most suggestive essay on the "Ultimate Aims of Agricultural Chemistry,"[2] that veteran author and critic of our science, Adolf Mayer, pointed to the unsolved basic problems of plant chemistry as the ones most deserving of investigation. It is to a few unsettled questions in this field that I would first direct your attention.

We are accustomed to determine the total water content of plants by some one of our prescribed methods of analysis, but towards understanding the nature of the aqueous fluids in the various tissues of our crops we have made but little progress. In certain cases, as in the analysis of the sugar cane, we make conventional estimates of the composition of the so-called "normal juice" and sometimes deceive ourselves by believing in the existence of such an entity. There occur in each plant not one but many saps, or juices,—as those of the vacuoles, the cytoplasm, the ducts, the nectaries, and other organs, each with its own peculiar composition and about which more information is desired.

By continuous percolation there has been obtained from the crushed

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[1] "Recent Contributions to the Chemistry of the Cotton Plant," by C. A. Browne, *J. Oil & Fat Ind.*, 2 87–96 (1925).

[2] "Letzte Ziele der Agriculturchemie," *Landw. Vers.-Sta.*, 109, 367–84 (1929).

blossoms of clover, alfalfa, and other plants an extract that has been assumed to resemble in composition the nectar of these flowers. The assumption is erroneous. It is only by applying the newly developed methods of microanalysis to the carefully isolated drops of nectar and other juices of the plant that we can arrive at a solution of the problem of their true chemical nature. When we have accurately determined the sugars, organic acids, enzymes, and other components of different floral nectars, we shall then be enabled, in our study of mutual chemical relationships, to investigate the transformation products of these substances when the nectar gathered by the bee is changed into honey. The microanalyst, by investigating the composition of the cellular juices of plants, will help greatly towards solving a number of important problems that relate to the chemistry of our crops and to the best methods for their utilization.

COMPARATIVE EFFECTS OF SOIL AND CLIMATE

The comparative effects of soil and climate upon the chemical composition of sugar beets, corn, and sorghum were subjects to which Dr. Wiley devoted a great deal of attention. We need more extensive investigations of this character in order to evaluate the respective influences of soils, fertilizers, cultivation, rainfall, temperature, altitude, sunshine, and other environmental factors on the yield and chemical composition of our different crops. A cooperative effort in this direction has recently been undertaken in Germany and a similar survey of the adaptability of the important soil and climatic regions of the United States for different crops might well be undertaken by our own agricultural experiment stations. A project of so vast a character can only be carried through by carefully planned cooperative effort.

It was realized nearly a century ago by early American investigators that a determination of all the constituents of a soil was of little value in drawing conclusions as to its productivity for crops under varying environmental conditions. Hitchcock, the first of the state geologists, had formed the conclusion in 1841 that the percentage composition of a soil could vary greatly without affecting its productivity. In this connection he remarked:

> If it should prove true, as I confidently think it will not, that there is a particular proportion of earthy ingredients most favorable to fertility***I apprehend that the same proportion will not produce the maximum of fertility in countries where the temperature and the amount of rain are different.[1]

A comparative vegetation experiment upon identical soils under different climatic conditions is the only method of determining the correctness of Hitchcock's statement. In 1895 Dr. Wiley initiated an extensive experiment upon the comparative fertility of forty-four virgin and cul-

[1] Final Report on the Geology of Massachusetts (1841), p. 32.

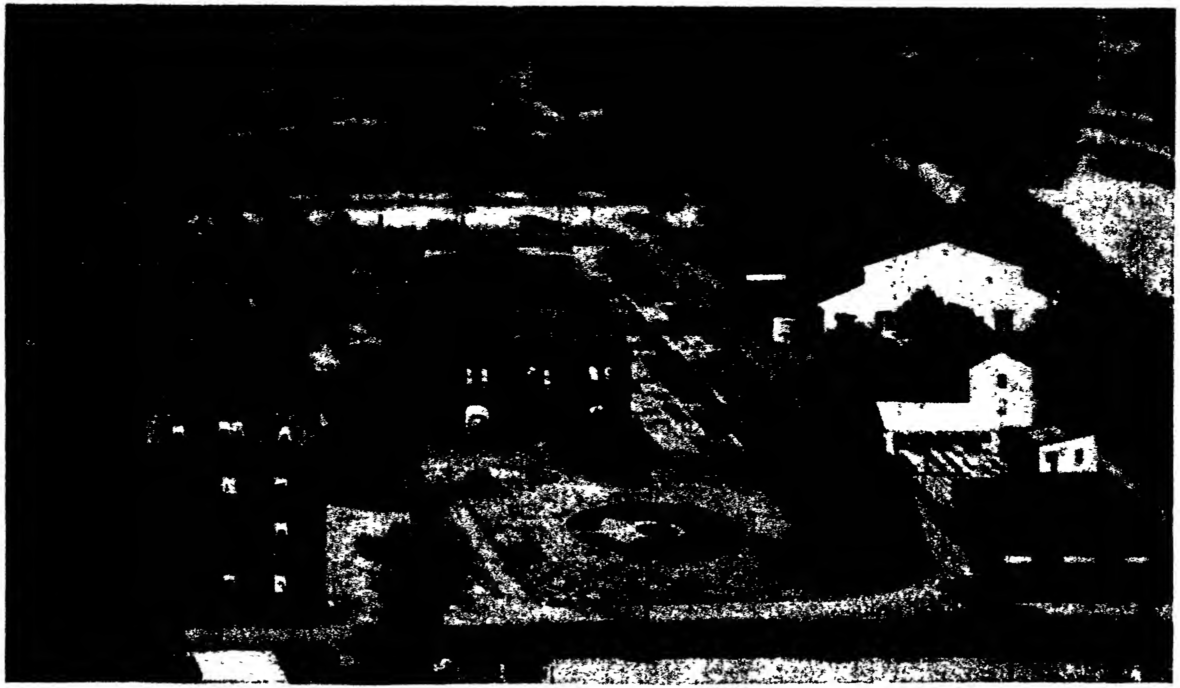
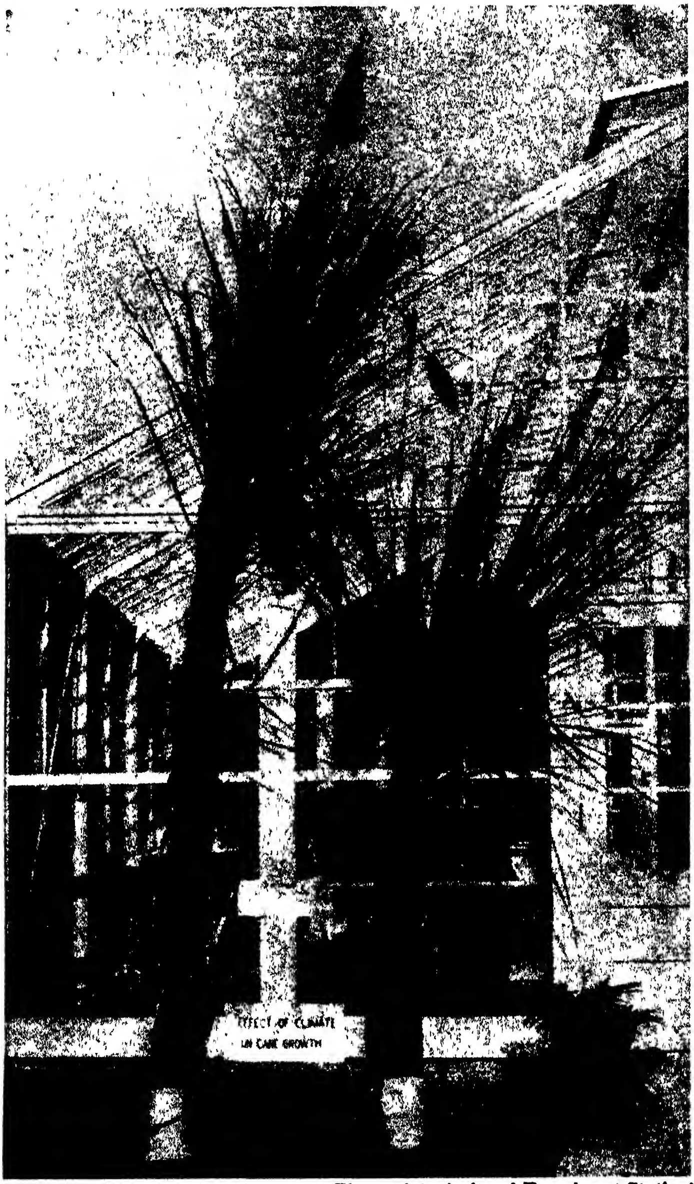


Fig. 1.—U. S. Department of Agriculture Buildings about 1900. Left, Main Building where A.O.A.C. meetings were held in 1885, 1886, 1887, and 1888. Center, Seed Division Building where A.O.A.C. meeting was held in 1889. Right, center, Vegetation Building and Yard where Dr. Wiley's pot experiments with soils were conducted



(Courtesy Hawaiian Sugar Planters' Agricultural Experiment Station)

FIG. 2.—EFFECT OF CLIMATE ON GROWTH OF SUGAR CANE.
H109 CANE GROWN WITH AMPLE FERTILIZATION ON "GOOD" MAKIKI SOIL:
AT MAKIKI—ON THE LEFT
AT MANOA—ON THE RIGHT

tivated soils from widely separated localities. This costly experiment, which was performed in a vegetation house and yard on a site now occupied by the East Wing of the Main Department of Agriculture Building, extended over a period of eight years, but the results, as in other unfortunate cases, were never published owing to opposition from another bureau.

In 1909 another soil-exchange experiment by the Bureau of Chemistry was begun by LeClerc and Yoder.[1] Three samples of soil five feet square and three feet deep, at College Park, Maryland; Hays, Kansas; and Davis, California, were dug up at each locality in 3-inch layers, sacked, exchanged with soils from the two other localities, and then replaced with layers in the same original positions. The three plots, thus prepared in each locality, were then sown with the same variety of wheat, and the resultant crops were harvested and analyzed. The experiment showed that the wheat grown on Maryland soil in Kansas contained over six per cent more protein than the wheat grown on Maryland soil in Maryland and that the wheat grown on Kansas soil in Kansas contained nearly eight per cent more protein than the wheat grown on Kansas soil in Maryland. The results indicated that under the conditions of this experiment the influence of climate upon the protein content of wheat was over three times stronger than that of soil.

The predominance of climate over soil in affecting the composition of crops may prevail in localities that are only a few miles apart. This is shown in a soil exchange experiment on the production of sugar conducted by Borden[2] in 1935 at the Hawaiian Sugar Planters' Experiment Station. Tubs of a fertile soil at Makiki and of a supposedly poor soil at Manoa, three and one-half miles distant, were planted with three different varieties of sugar cane, which were allowed to grow at the two localities for 14 months. The canes were then cut and topped, the stalks weighed and crushed and the juice analyzed. The results showed for the Makiki soil that the canes grown at Makiki produced 7.9 pounds of sugar and those grown at Manoa only 2.9 pounds of sugar; while for the Manoa soil the canes grown at Makiki produced 10 pounds of sugar and those grown at Manoa only 2.6 pounds of sugar. In this experiment the influence of climate was over 7 times and that of variety nearly 3 times stronger than that of soil. As far as productivity is concerned the supposedly "poor" Manoa soil was the better. The 150 inches more of rain and the 40 per cent less of sunshine at Manoa seem to be the climatic factors chiefly responsible for these results. The striking difference in the growth of the canes at the two places is shown in the accompanying photograph (Fig. 2).

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[1] 8th Intern. Congr. Applied Chem., Vol. 26, p. 137.
[2] "Cane Growth Studies, the Dominating Effect of Climate," *Hawaiian Planters' Record*, 40, 143–56 (1936).

The dominance of climate over soil may influence also the production of those more subtle ingredients of crops that affect our senses of taste and smell. Grasse in France, because of its sheltered location, nearness to the sea, favorable rainfall, adequate drainage, bountiful sunshine, and other delicately balanced conditions, has proved to be an ideal place for the production of floral essences. The same variety of flowers, grown on similar soils only fifty miles away, yields perfume in less amount and of inferior grade.

We do not yet know the quantitative effect of different climatic factors in affecting the growth and composition of crops, although rough formulas have been developed in some cases for making forecasts of production from measurements of temperature, rainfall, and sunshine. Such methods require a long statistical study for their perfection.

Adverse climatic conditions have been counteracted in some cases by a modified system of fertilization, although this method must be used with caution because of the varying effect of fertilizers upon different crops under different conditions. Thus Borden[1] found, in the experiments previously cited, that the POJ 2878 variety of sugar cane was adversely affected by heavy applications of fertilizer under the climatic conditions of Manoa, whereas similar applications had no injurious effect upon the cane grown under the more favorable climatic conditions of increased sunshine and diminished rainfall at Makiki. The chemical explanation of such occurrences is not always apparent. The excessive leaching away of the water-soluble constituent of a complete fertilizer may possibly create a situation similar to that in the experiments of Lagatu and Maume,[2] who found that under certain conditions plots treated with an unbalanced fertilizer, in which one necessary ingredient was lacking, gave lower yields than check plots that had received no fertilizer at all. Determinations of the mineral constituents in the leaves of crops were found by these experimenters to give an indication of fertilizer needs, a procedure somewhat analogous to that followed in the Neubauer method with rye seedlings. The work of Lagatu and Maume has received favorable notice from several commentators,[3] but additional investigations are needed before conclusions based upon leaf analyses can be accepted as a reliable guide for the fertilizer requirements of various crops on different soils.

LAW OF THE MINIMUM

The work of Lagatu and Maume has indicated again the need of reinterpreting the so-called law of the minimum, first announced a century ago by Carl Sprengel[4] and afterwards wrongly accredited to Liebig. Ac-

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[1] "Cane Growth Studies," *Hawaiian Planters' Record*, 40, 155–6 (1936).
[2] *Compt. rend. acad. agri. France*, 13, 437–55 (1927).
[3] See especially the article of Walter Thomas, "Foliar Diagnosis; Principles and Practice," *Plant Physiol.*, 12, 571–99 (1937).
[4] "Die Bodenkunde". Leipzig (1837), pp. 303–4.

cording to Sprengel, if only one of the elements essential for growth be lacking, the plant will not thrive, though all the other necessary constituents occur in abundance. Sprengel also announced that if a water-soluble element be present in excess the result is equally unfavorable and that between these two extremes was the level of the optimum at which the crop made its most favorable growth. Recent proposals to substitute for the term "law of the minimum" the designation "law of the optimum" are therefore directly in line with Sprengel's original concept.

As emphasized by Liebig in the later editions of his classic treatise[1] on agricultural chemistry, the "law of the minimum" applies not to one plant nutrient alone but to all. Wollny extended the applications of the rule so as to include the physical as well as the chemical conditions of the soil until finally today the "law of the minimum" has acquired a universal significance with inclusion of sunlight, rainfall, temperature, and all other climatic and environmental influences. The working of the law, moreover, differs with each crop and with each variety of crop. As the investigator delves more deeply into the subject and discovers the interplay of so many hitherto unrecognized growth factors the problem of the minimum, apparently so simple in its first conception, is seen to acquire an almost baffling complexity. Nolte in a very illuminating essay upon the minimum theory made the following significant remark:

> The curves of crop yields are very similar to the curves of a catalytic chemical reaction. An advancement in agricultural chemistry is only to be gained when we begin to study plant nutrition from that viewpoint and to apply the laws of catalysis to investigating the manifold factors that influence the yields of crops under the slightest alteration of environmental conditions.[2]

Sprengel applied his theory not only to the major essential constituents of soils but to what are now termed trace elements, of which he was the first to indicate the possible importance.[3] His rule of avoiding the "zu wenig" and "zu viel" is well exemplified by recent work with the trace element boron, a complete lack of which provokes diseases of the sugar beet, turnip, and cauliflower, while a slight excess is equally disastrous, as was shown in the emergencies of twenty years ago when potash salts, contaminated with borax, were used as a fertilizer. The optimum requirement for boron lies within exceedingly narrow limits, the demarcations of which for different soils and crops have not yet been definitely established. The same observation applies to other minor elements such as zinc, copper, and manganese. Some extremists believe that plants and animals require for their best development traces of every member of the periodic table, and powdered preparations of sixty or more trace elements have recently been proposed as adjuncts to the ordinary fertilizer mixtures. In the present conflict of opposing views on this and other

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[1] "Die Chemie in ihrer Anwendung auf Agricultur und Physiologie," 7th ed. Part II, p. 227 (1862).
[2] Otto Nolte, "Das Minimum," *Landw. Vers.-Sta.*, 109, 317–42 (1929).
[3] "Lehre vom Dünger," 2nd ed., p. 49 (1845). Leipzig.

questions a certain amount of conservatism is required. In matters of doubt we can do no better than follow Dr. Wiley's favorite Latin maxim, "Medio tutissimus ibis," or "The middle path is the safest."

Proposals have been made to increase the content of nutritive mineral elements in crops by special intensive methods of fertilization. The mineral content of crops can be modified within certain limits in several ways. Different varieties of wheat, potatoes, and other crops vary in their capacity for assimilating different elements from the soil. The production of crops with a preponderance of certain mineral nutrients might be advantageous for specific purposes provided there were not corresponding losses of other valuable constituents. There are, however, so many factors that influence the yield and composition of crops, such as differences in soil, cultivation, altitude, rainfall, temperature, and sunshine, that a special mineralization formula adapted to one locality might be highly detrimental in another.

It is a strange anomaly that in their study of the human, animal, plant, and mineral worlds, chemists have usually selected problems in the inverse order of their immediate concern. In the regulatory field laws were passed first to control the purity of fertilizers, then followed laws governing the quality of cattle feeds, and finally standards were formulated for the sale of human foods. The same sequence has been followed in the field of research. The relative abundance of fifty elements in the rocky crust of the earth has been carefully tabulated upon the basis of thousands of analyses, with an apparent exactitude to the eighth decimal in the case of the rarer elements, while only rough compilations have been made of the abundance of some twenty or thirty elements in the average soil. In the case of plants the obsolete tabulations of Wolff, the most complete that we have, give the abundance of only nine of the common mineral elements occurring in crops. As for the composition of the mineral matter occurring in animals and man, only a few very rough tabulations of the more important elements are available, exact information upon the relative abundance and distribution of the minor constituents being almost wholly lacking. The filling of these gaps in our knowledge is one of the great unsolved problems of agricultural chemistry.

In a conversation a few years ago with the late Fritz Haber, whose work on nitrogen fixation has done more to revolutionize agriculture than any chemical discovery of the past half century, he made the significant remark that in experiment station research too much attention had been given to fertilizer technique and too little to the breeding of new crop varieties with special productive endowments. He expressed the thought that great as had been the contributions of chemistry to agriculture, future progress would probably be more in the field of genetics. This opinion, so thoroughly characteristic of Haber's lack of bias, does not eliminate the chemist, however, from the prominent part which he is

destined to play in the future development of agriculture. In the breeding of new varieties of useful crops of higher content in sugar, starch, protein, oil, tannin, nicotine, and other valuable constituents genetics must go hand in hand with chemistry. The improvement of the sugar beet from its original five per cent to its present eighteen or twenty per cent of sugar is as much a triumph of chemistry as of plant breeding.

TABLE 1.—*Approximate percentages of elements (excluding oxygen) in the mineral matter of rocks, soils, food plants, and man*

ELEMENT	IGNEOUS AND SEDIMENTARY ROCKS[1]	SOILS[2]	FOOD PLANTS[3]	HUMAN BODY[4]
	per cent	*per cent*	*per cent*	*per cent*
Silicon	51.96	71.63	2.05	—
Aluminum	15.14	13.74	trace	—
Iron	9.48	6.86	1.58	0.13
Calcium	6.85	1.02	8.44	42.24
Potassium	4.84	3 02	52 85	8.34
Sodium	5.16	1.08	5.56	6.03
Magnesium	3.90	.68	6.06	1.32
Titanium	1.16	1 06	trace	—
Phosphorus	.24	.12	16 77	23.85
Manganese	.17	.12	trace	trace
Sulfur	.10	.10	3.03	13.14
Barium	.09	.17	trace	—
Chlorine	.08	.07	3.50	4.84
Chromium	.07	.01	trace	—
Fluorine	.05	.06	trace	.06
Undetermined (zinc, copper, iodine, cobalt, boron, etc.)	.71	.26	.16	.05
Total	100.00	100 00	100 00	100 00

[1] Compilation of many analyses by Clarke and Washington, Professional Paper 127, U. S. Geol Survey (1924).
[2] Twenty-six analyses of various soils by Robinson, Professional Paper 122, U. S. Dept Agr (1914)
[3] Twenty analyses of common food plants, Wolff's Aschenanalysen (1880)
[4] Average of two compilations by Sherman (Chemistry of Food and Nutrition, 4th ed.) and Bertrand, *Bull. Soc. Science d'Hygiene Alim.*, **8**, 53 (1920).

A question that I heard frequently asked in Germany some years ago was, "Why do our fields not respond to fertilizers as they did before the war?" "Invasion of the soil with nematodes," was one reply. "Derangements of the soil by bad tillage," said another. "Excessive use of synthetic fertilizers," answered a third. "Failure to supply sufficient humus," was the response of the late Professor Löhnis of Leipzig, and his verdict was probably correct. Mineral fertilizers have been found to give their best response only in the presence of a sufficient amount of decaying organic matter. Hence the intensive research being given by German investigators to the chemistry of composting. It is a subject to which more attention should be devoted by agricultural chemists in the United States.

DETERIORATION OF AGRICULTURAL PRODUCTS

The deterioration of our agricultural products by micro-organisms, enzymes, and atmospheric influences is being actively studied by chemists at present. The problem in its relation to the storage of perishable commodities is of tremendous economic importance, the losses from rancidity, decay, impairment of flavor, and other causes amounting each year to many millions of dollars. In addition to the three agencies just mentioned there is a fourth factor of very obscure nature that has been generally overlooked. This is the deterioration that results from chemical reactions within the product itself. Sirups, honey, and other food products darken as a result of slow combinations between reducing sugars and amino acids. Tins of canned molasses, although sterile and preserved from contact with the air, sometimes burst as a result of chemical decompositions within the product. Disagreeable flavors and odors are generated and losses in nutritive value occur. The chemical reactions involved in these spontaneous changes are not fully understood, and they constitute a problem that deserves more attention on the part of agricultural chemists. Periodic analyses, at long intervals, of type samples prepared in large quantity and stored under various conditions offer one of the best means of attacking this difficult problem.

Spontaneous decomposition proceeds in some cases slowly and in others with such rapidity that the product heats, carbonizes, and even bursts into flame. The frequently observed spontaneous ignition of an oily rag is attributed to the rapid absorption of atmospheric oxygen by unsaturated fatty acids but the spontaneous combustion of a haymow, a phenomenon responsible for annual losses of many millions, presents vastly greater difficulties of explanation. A slow period of initial heating, due to enzymic and bacterial activity, is followed by a short period of accelerated heat production that far exceeds the death-point of any micro-organism or enzyme. It is probable that in the incipient anaerobic stage of fermentation within the haymow, unsaturated compounds are produced, which later upon accidental exposure to the air absorb oxygen with such avidity that the heat rises to the point of ignition.[1] The detailed steps of this reaction, however, are still an unsolved problem. Long investigations by the Bureau of Chemistry and Soils show that the exact balance of temperature, moisture, air exposure, and other conditions necessary to produce spontaneous ignition of a haystack are not easy to duplicate in a practical experiment. Great as are the annual losses from spontaneous ignition, the less spectacular losses from destruction of nutritive matter and decrease in digestibility, when the hay heats but does not ignite, are many times greater.

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[1] For further details consult Tech. Bull. No. 141 of U. S. Department of Agriculture "The Spontaneous Combustion of Hay."

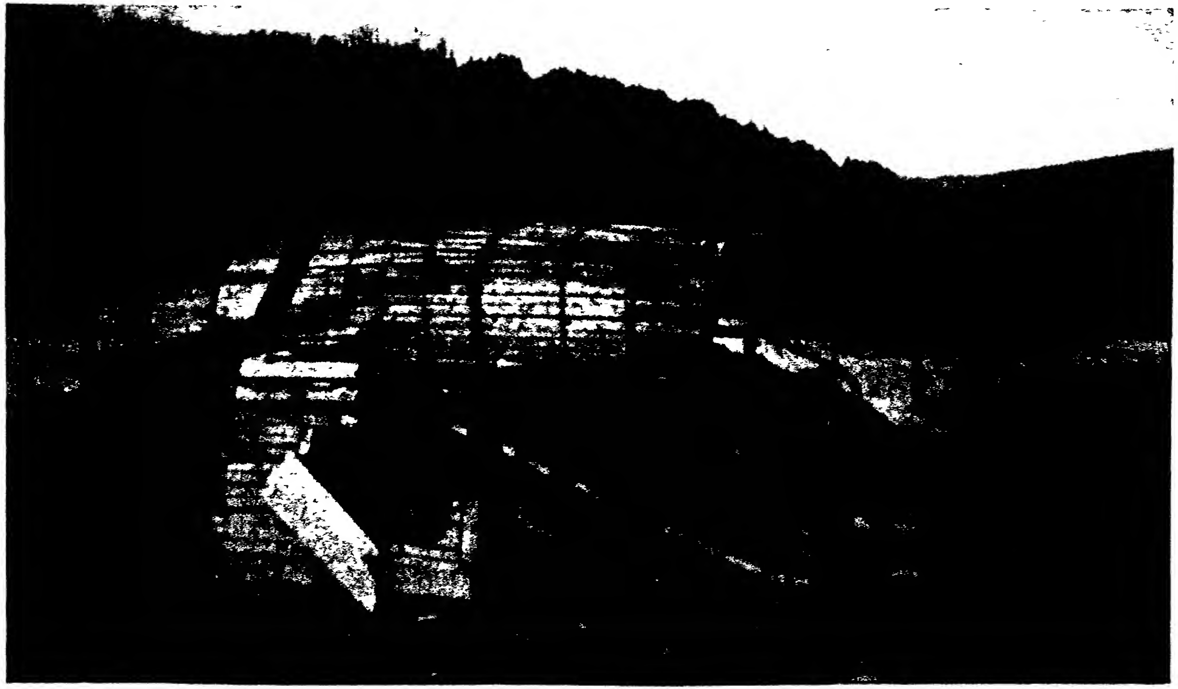

Fig. 3.—Destruction of Vermont barn and silo by spontaneous ignition of hay following the flood in November, 1927, on the Winooski river

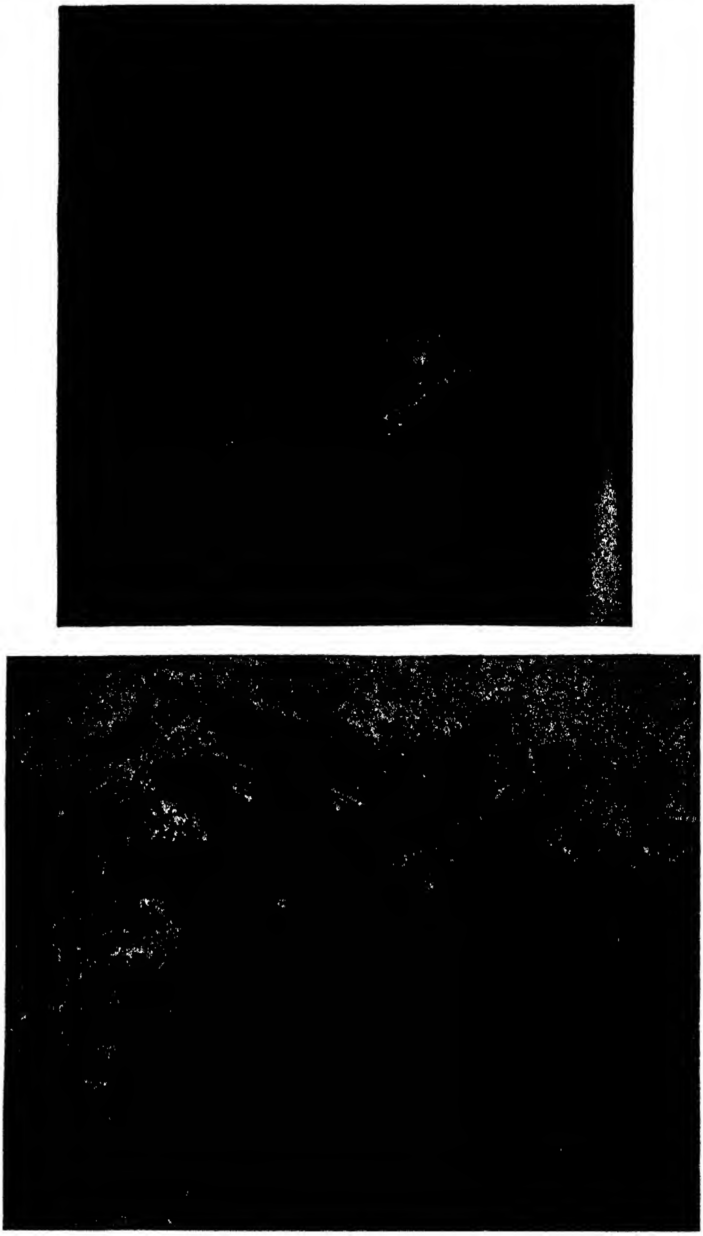

FIG. 4.—HOT POCKET IN HAYMOW OF BARN DAMAGED BY SPONTANEOUS COMBUSTION NEAR ANNAPOLIS, MD.

LOWER, POCKET IMMEDIATELY AFTER OPENING

UPPER, SAME POCKET BURSTING INTO FLAMES 13 MINUTES AFTER OPENING

ECONOMIC CONSIDERATIONS

A wider class of agricultural-chemical problems, in which Dr. Wiley was especially interested in his later years, relates to questions of national economic significance. While such problems fall outside the range of our ordinary laboratory operations, the agricultural chemist, if he is to keep abreast with the times, must give them due consideration and contribute his share to their solution. In many cases he alone is the one most competent to give the answer. Consider for example the implications involved in answering the question, "Is the sugar beet an economic crop?", about which politicians in several countries have so much debated. Judged alone by the comparative costs of producing beet sugar in temperate zones and cane sugar in the tropics the sugar beet appears to be a very uneconomic crop. But when we consider the great enrichment of the surface soil produced by growing a deep-rooted crop such as the sugar beet, the much greater productive power which the beet gives to the soil for succeeding rotation crops, the great stock feeding value of beet pulp and molasses, and the numerous indirect benefits to domestic labor and industry, the balance of opinion will be that the sugar beet is an economic crop wherever it can be successfully grown. The indirect benefit or injury of farm practices are subjects that the agricultural chemist must always consider.

The time is not far distant when with the growing population of our country and the final occupation of all available farm land, increasing attention must be given to the maximum production of particular crops in the areas best suited to their cultivation. It is then that the agricultural chemist will be called upon to answer such questions as: "What soils, fertilizers, and methods of culture will give the highest economic yields of sugar, starch, cellulose, protein, rubber and oil?" "To what extent are we as a nation justified in trying to produce at home all our domestic needs of these and other agricultural commodities?" "What is the maximum population that a given area of farm lands can support?"

Such questions have been propounded before and agricultural chemists even at the risk of venturing upon the hazardous ground of prophecy, have not hesitated to submit their replies. Thus Professor James Finlay Weir Johnston, whose famous book on agricultural chemistry is still running strong in its twenty-first edition, visited the United States and Canada in 1849 and 1850 and among many other queries was asked how great a population the province of New Brunswick was capable of supporting. Like a true Scotchman he based his estimate upon the productivity of New Brunswick for oats. He conjectured that the average person living entirely on oatmeal would require 40 bushels of oats annually for his proper maintenance. By multiplying the average production of oats in New Brunswick per acre by the estimated potential farm acreage of the province and deducting from this the calculated consumption required by farm animals he obtained the total number of bushels available

for human consumption, which divided by 40 gave him a figure of 4,200,000 as the possible population that New Brunswick was capable of supporting.[1]

A half century later Dr. Wiley, in an address which he gave on "The Dignity of Chemistry," ventured to make a prediction of how things might be in 1976 on the two hundredth anniversary of our national independence. He commented as follows:

> May I drop for a moment the role of chemist and assume that of prophet? Our country will have then about 225,000,000 inhabitants. Our foreign export trade will amount to more than $5,000,000,000 annually. The revenues and expenditures of our Government will each reach the annual sum of $4,000,000,000. The average yield of wheat in the United States will be nearly 25 bushels per acre, and the average yield of other field crops be proportionately greater than now.
>
> Diversified manufacturing industries will flourish in every part of the country, thus distributing population and encouraging agriculture. The product of a day's labor will be double that of today, thanks to new processes, improved machinery and greater skill. The condition of the artisan and the laborer will be greatly ameliorated, and the principles of the trust, which now help chiefly the capitalist, will be extended to include the working man as well. The laborer will not only have a larger daily wage, but will also share in the legitimate profits of the business.
>
> The advancement of chemical science will not only make the fields more productive and more easily tilled, but will also teach how their products can be more economically and easily consumed. Good roads will lead everywhere and the horse be relegated to the museum and the stable of the sportsman. New sources of energy will take the place of coal and gas, and this energy will come from the winds and the rains. The sun directly and indirectly will monopolize the power of the country, working through evaporation and precipitation and by means of electricity or some more useful force.
>
> By a general comprehension of the principles of nutrition, food will be more wholesome and more potent. The general acceptance of the principles of hygiene will make the average life of man longer and his usefulness more fruitful. Man will not only live longer, but he will be happier and practically free from the threats of enzymic, contagious, and epidemic diseases.[2]

It may be assumed that Dr. Wiley attributed many of these predicted advances in attacking the unsolved problems of 1901 to the future work of the chemist. Prophecies, however, are as apt to be understated as overdrawn and we, who now look back upon his predictions, will smile at many of his conservative estimates. The foreign exports of the United States in 1920 exceeded $8,000,000,000. The expenditures of our country passed the $4,000,000,000 mark in 1925 and more than doubled this amount in 1936. Dr. Wiley's predictions as regards diversification of manufactures, introduction of new processes, increased output of labor, larger daily wages, advancement of chemical science, more economical utilization of products, better roads, relegation of the horse, lengthening the average of human life, and the eradication of contagious diseases have

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[1] See Trueman's "Early Agriculture in the Atlantic Provinces." Moncton, 1907, pp. 26–29.
[2] "Twenty-fifth Anniversary of the American Chemical Society." Easton, 1902, pp. 163–4.

already been largely realized, while his other prophecies about number of inhabitants, average yield of wheat per acre, and utilization of new sources of energy are less certain of fulfilment.

We have advanced considerably in the perfection of statistical methods since Professor Johnston in 1850 and Dr. Wiley in 1901 made their predictions. More recently Dr. O. W. Willcox (also an agricultural chemist although he prefers the title of agrobiologist), basing his calculations on the growth factors of Mitscherlich and others, has ventured to prophesy as to the "perultimate" yields of several crops and gives such estimates as 225 bushels of corn per acre, 171 bushels of wheat, and 1550 bushels of potatoes as the *ne plus ultra* of production.[1] While these figures have been partially approached in a few rare instances, the cases are due to a combination of exceptionablly favorable conditions and such enormous yields are far beyond the limits of general economic attainment. A careful study of all the factors that favor phenomenal productivity would no doubt lead to results of considerable value. As to what the future may bring forth we can only say that everything hinges upon the limits of yield and composition that can be attained by plant breeding and upon future progress in methods of culture, fertilization, and control of pests.

Many other unsolved problems of agricultural chemistry offer tempting subjects for consideration but lack of time obliges me to pass them over. I have selected for the present occasion only a few types of such problems as interested Dr. Wiley in the course of his long productive career. They relate to subjects which we used to discuss together as neighbors and as companions during our morning walks down Connecticut Avenue.

Dr. Wiley's famous crusade against food adulteration was of such a spectacular nature that it overshadowed his activities in agricultural chemical research. It should not be forgotten, however, that the reform movement, which he initiated, had its origin in research and that in the midst of his efforts to secure the passage and enforcement of a pure food law he was continually promoting investigations in the field of pure science. May his optimism and breadth of vision long continue to inspire the members of our Association and direct our steps towards the goal to which at these annual meetings he always pointed the way.

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[1] "ABC of Agrobiology." New York (1937), p 221.

PRESIDENT'S ADDRESS*

By C. C. McDonnell (U. S. Food and Drug Administration, Washington, D. C.)

An unwritten law of this Association apparently demands that the incoming president shall make an address. This is indicated by the past history of the Association and quite forcibly by the fact that provision is made for it on the program without consulting this official. Fortunately for him, however, the subject upon which he may speak before the Association is left to his own choosing, and in reviewing the addresses of former presidents I find that they have covered a wide range of topics. I regret that I am not in a position, as has been the case with many of my predecessors in this office, to present to you a paper embodying the results of research in a field relating closely to this Association's work, but being engaged in regulatory work and having no opportunity for fundamental research I shall speak on a subject of a more general character. Since the problem of insect control is a live one at the present time I have selected as my subject, "The Insect Menace and The Rôle of Chemistry in Combating It."

The history of insect life reads like a romance. It has been estimated that many species existed in a high state of development as long ago as one hundred million years or more and, as indicated by fossil remains that have been discovered, have not changed so far as it can be determined during that time. Many fossil remains of roaches, apparently the same as those we have today, have been found in coal deposits, showing that they existed in great numbers during the carboniferous era. Hence, if we consider that the age of man is less than one million years (as has been estimated by paleontologists), and if, in addition, it is realized that there are one hundred or more generations in the life of the insect to one of man, we see what a tremendous advantage the insects have had in adapting themselves to life on the earth. They possess characteristics that man does not possess and cannot acquire, such as the ready adaptation to environment; rapidity of multiplication (no methods of birth control practised); short period of infancy; power of flight in many species; power of concealment due to their small size and color adaptability; protection afforded by hibernation; superior methods of defense,—many species being protected by a defensive armor and other anatomical advantages; feeding habits; etc. There is no "old age" with insects,—when their work is done they die.

The opinion generally held that in our fight against insects we are pitted against creatures of low intelligence is far from correct. Nowhere in the animal kingdom does the self-sacrificing cooperation of its individuals exist to the degree that it does in the more highly developed species of

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* Presented Monday afternoon, November 1, 1937.

insects, such as the ants and the honey-bees. As expressed by some writers they enjoy what, to us, may be considered as only a "social ideal." Take, for example, the ants. The mother ant or queen lives under the protection of the home, usually underground, which she never leaves, is fed and cared for by the workers,—once fertilized always fertilized,—and her only function seems to be to lay eggs, which in some species may be at the rate of many thousands a day. These, and the young when hatched, are cared for by others of the colony.

Another class of insects that possesses remarkable properties of adaptation to environmental conditions is the aphids. Some species undergo a complete change in their method of reproduction according to the weather. For example, during the summer months they give birth to living young, producing several generations, the number depending upon the length of the season. With the exception of the last generation in the fall all of these are fertile females, some winged and some wingless, and they in turn also produce living young females. With the approach of cold weather both males and females are produced. The females of this generation deposit eggs on the branches and twigs; the eggs remain over until spring when they hatch, and again all the young are fertile females.

According to the many accounts of insect scourges that are reported in early writings, insect pests have harassed man in all ages. Notwithstanding such knowledge, there has until more recent years been a tendency, particularly on the part of the general public, to ignore or to look with indifference upon the insect menace. This has been due in part, no doubt, to the fact that the term "bug" carries with it a contemptuous significance. The entomologist of earlier days was frequently subjected to derision, being called "The Bug Catcher" and pictured as an unattractive, bewhiskered individual chasing butterflies with a net attached to a pole. Even the eminent Dr. Howard has stated in his writings that he felt keenly in his younger days the ridicule that was heaped upon the "bug catcher." Yet he recognized the great economic importance of the insect problem and dedicated his life to the work, becoming one of the world's leading authorities on entomology. As stated by Dr. A. F. Woods in an address on his life and work, "Dr. Howard is credited more than any other one man with awakening the United States to the peril of insect pests. He has dramatized man's struggle against harmful insects as a war without quarter for possession of the world's food supply."

It is not possible to express accurately in dollars the loss to our agricultural industry due to insects, owing to the many factors that enter into the making of such an estimate. In fact a reduced crop may mean a higher return to the grower due to a higher unit price, but it means a lower profit to the railroads or other carriers and to the distributors, and an increased cost to the consumer. Dr. Howard has stated that insects destroy ten per cent of our crops before they are harvested and that the

damage to stored grain and milled products represents a loss of five per cent of their total value. He has also stated that insect ravages nullify the labor of 1,000,000 men annually. Another writer estimates that the economic loss to farmers of the United States due to insects is greater than the cost of educating our children. To this should be added the damage to forests and forest products, to our clothing and our furniture, to the livestock industry, and to man himself through diseases that are insect-borne,—to say nothing of the great annoyance to his comfort. I have not considered in this paper the problem of plant diseases caused by fungi and bacteria, the losses from which are next in importance to the losses caused by insects.

As late as 1931 Dr. Howard stated that the insect menace is increasing under present conditions and the noted Belgian scientist and author, Maeterlinck, stated in his writings that one day insects would be the successors of mankind upon the earth. The famous surgeon, Dr. William Mayo, said in an address delivered before the meeting of The American Chemical Society in St. Louis in 1928, "The easily recognized and obvious enemies of man,—beasts and serpents, storms and earthquakes,—are not numerous, but his less conspicuous and his microscopic enemies exist by the millions. The fight between man and parasites is a battle to the death for food, because insects, protozoa, and bacteria have essentially the same food necessity and were at home on the globe millions of years before man." Dr. Mohler, Chief of the Bureau of Animal Industry, stated before the Agricultural Committee of the House of Representatives that unless a comprehensive control program is inaugurated the future food supply of the country may be seriously menaced.

We often hear older people say, "Why is it the insect problem is so much more serious now than when I was a boy? We did not hear so much about it then." As a matter of fact, insects are much more numerous in this country and do infinitely more damage now than when some of us were lads. This is due to a number of causes. Man has created conditions peculiarly favorable to their distribution and increase. Every advance that speeds transportation, whether by air, land, or water, lends aid for the spread of these pests from one region to another, and the intensive culture that accompanies civilization furnishes them with an abundance of food and encourages their multiplication. The majority of our most destructive insect pests in this country are of foreign origin, and many of them have appeared during this generation. The gypsy moth came from Europe, as did also probably the codling moth; the brown-tail moth from Holland; the cotton boll weevil from Mexico in 1893; the Japanese beetle, as the name indicates, from Japan in 1916; and the corn-borer from Hungary. The Colorado potato beetle did not occur in the eastern part of the United States until about 1875; the San José scale appeared in the nineties; and the Mexican bean beetle, which is by far the most serious

insect enemy of beans, was not known east of the Mississippi river until 1920. It now infests most of the bean-growing areas throughout the United States.

As stated by Dr. Howard in 1931, "We are awakening from our apathy concerning the insect menace." The fact that we are becoming insect conscious is impressed upon us continuously in our daily reading matter. Over five thousand papers and books on entomological subjects are published annually and, in addition, innumerable articles appear daily in our newspapers and magazines. It is also manifest in our music. At the recent meeting of The American Chemical Society held at Rochester, New York (the second largest meeting in the history of the Society), the musical program given by the orchestra preceding the president's address included "The Flight of The Bumble Bee," "Mosquito Dance," "The Bee," and selections from "Fire-Fly."

In the earlier days insect plagues were looked upon as visitations from Providence, and days of prayer were set aside by church and state officials to call on God to allay the ravages of insects. In the spring of 1848 hordes of crickets attacked the crops in the fields near Salt Lake City where the Mormons had recently established a colony. The people assembled around their fields and prayed, and almost immediately, it is recorded, thousands of sea gulls swarmed overhead, alighted in the fields, and devoured the crickets, thus saving the greater part of the crops. The grateful Mormons erected a monument to the sea gull, which stands just outside the gates of the Temple in Salt Lake City. As late as 1875 the Governor of Missouri appointed a day for fasting and prayer on account of the ravages in that state of the Rocky Mountain locust.

It is impossible to tell when the fight against insects was begun, but it is probable that control methods were first directed against those that pestered man himself. A professor in one of our leading universities has stated that extended researches have developed the fact that man took to the wearing of clothes for the purpose of protecting himself from annoyance by insects. Methods for destroying insects attacking vegetation were started, no doubt, soon after the cultivation of plants began to attract serious attention. In his writings in the first century, Pliny speaks of mildew as "that greatest curse of all corn" and states that "if branches of laurel are fixed in the ground it will pass away from the field into the leaves of the laurel." He refers to insect pests attacking grain and recommends steeping the seed in wine or mixing bruised cypress leaves with it. Pliny also quoted Democritus, who lived between 400 and 300 B.C., as recommending "sprinkling the plants with Amurca of olives without salt to prevent the blight from attacking them and to destroy worms adhering to the roots." Another method recommended was to carry a bramble frog at night around the field and then bury it in an earthen vessel in the middle of the field. Later various applications to the plants were recom-

mended, but the materials suggested as being most effective were generally those having the vilest smell or most offensive taste.

The next step in control methods was by the use of chemicals. Sulfur, which was known to the ancients, was one of the first to be recommended. Cato, writing about 200 B.C., states that "one method of destroying the 'vine fretter' was to fumigate the trees with smoke from a mixture of Amurca of olives, sulfur and bitumen for three days in succession." References to the use of arsenic as an insecticide are also found in very early writings. Pliny suggests that if the fruit is falling from the vines, or the grapes rotting, they should be sprinkled with sanderach (a natural arsenic sulfide).

Several of our other present-day insecticides were used in some form more than a hundred years ago. Tobacco dust was recommended in France for use against plant lice in 1763. Pyrethrum powder has been in use for more than one hundred years. Arsenic and honey mixture for ants was recommended about 200 years ago. Marco Polo, in his writings at the end of the 13th century, mentions the use of crude mineral oil for anointing camels that have the mange. Peter Kalm, a Swedish scientist who visited this country in 1748–1749, in his book, "Travels in North America," refers to the insect pests he encountered and the remedies used by the people here to combat them. Among the materials mentioned are a decoction of "white mullein," to be applied to the wounds of cattle infested with worms; burning sulfur for bedbugs; an extract of hellebore root to be used when the children "are plagued with vermin"; and shavings and chips of red cedar distributed in clothing to protect it against being worm eaten. He states also that bureaus and chests made of red cedar are used for the same purpose, but adds, "It is only useful for this purpose as long as it is fresh."

But little progress in the methods for the destruction of harmful insects was made in this country until about 1860, when the currant worm had been introduced into the Eastern States and the Colorado potato beetle into the Western. Between 1860 and 1870 Paris green, which was being used as a paint pigment and therefore was readily available, was tried against the potato beetle. The results were so satisfactory that for many years it was the standard treatment for potatoes to control this pest, and it is still used to the extent of four to five million pounds annually for this purpose and for other garden crops. However, it was nearly 30 years later before organized investigations were directed toward the development of more effective means of insect control and better insecticides. With the growing diversity of our population and the introduction of new pests the problem became more urgent.

After the passage of the Hatch Act in 1887 and the establishment of the State Agricultural Experiment Stations great impetus was given to the use of insecticides. The Federal Government had already appointed en-

tomologists, who were actively engaged in the study of control methods, but it was not until 1896 that it recognized the important rôle chemistry was to play in this problem and appointed a chemist to work in this field, the late Dr. J. K. Haywood, who became Chairman of The Insecticide and Fungicide Board soon after the passage of the Insecticide Act and the 34th President of this Association. Dr. Haywood, working under Dr. Wiley in the then Bureau of Chemistry, was a pioneer and leader in this field, a man of whom our Secretary, Dr. Skinner, in a biographical sketch of his life and work published in the *Journal* of this Association, wrote "It may be said that he and his colleagues working in the Association of Official Agricultural Chemists created an insecticide chemistry."

I shall not attempt to review the entire history of insecticide development, but merely point out a few of the more outstanding achievements. In 1892 lead arsenate came prominently before the public as an insecticide for the gypsy moth, against which it is particularly effective. In a short time it largely replaced Paris green as an insecticide for plants, and for many years it has been used more extensively than any other arsenical for orchard pests. For the past ten years the amount used annually has averaged about 30,000,000 pounds.

At about the time the gypsy moth started to attract serious attention, another destructive insect, the cotton boll weevil, made its appearance in Texas. Gradually it extended its area of infestation until now it covers practically the entire cotton-growing districts. As a result of a study of methods for the control of this insect calcium arsenate was develpoed about 1920 and was found to be the most effective insecticide for use on cotton. Its use increased rapidly, and at the present time the annual consumption approximates that of lead arsenate. The only other arsenical compound that is used extensively as an insecticide is white arsenic. Owing to its caustic qualities it cannot be used on foliage, but it is used in large quantities in poisoned baits for the killing of grasshoppers and in the form of an alkaline solution for the dipping of cattle to eradicate cattle ticks. Two to three thousand tons are used annually for these purposes. It is thus seen that arsenical preparations for the control of insects are used in excess of one-half of a pound every year for each man, woman, and child in the United States. This large quantity, much of which is applied to our fruits and vegetables, is causing much concern, and rightly so, to our food chemists, health authorities, and the general public. In addition to the arsenicals, the other poisons of various kinds used on our crops and animals and in our homes amount to many millions of pounds annually.

At present inorganic materials dominate the market, but owing to the health hazard involved from the presence of toxic residues on sprayed fruits and vegetables and the restrictions being placed upon the marketing of these products by food control and public health officials the insecticide

industry is undergoing a marked change. Extensive research work is being done by the Federal and State Governments and other agencies to develop insecticides that will minimize this health problem. The substitution of synthetic organic compounds offers the most promise. Some progress has been made in this field, but its possibilities are limitless. The principal compounds of this character used at the present time are naphthalene, paradichlorobenzene, certain aliphatic thiocyanates, chloropicrin, and more recently ethylene oxide and ethylene dichloride. The two compounds last mentioned, mixed with carbon dioxide, are now being used on a large scale for the destruction of insect pests in food products, stored grain in elevators, and for the fumigation of flour and feed mills. They are also replacing to a large extent the highly inflammable and explosive carbon disulfide, which has been used extensively for these purposes for many years, and hydrocyanic acid gas, which is so toxic to man.

The most recent development in the field of new products is the use of rotenone-bearing plant material and extractives, which are highly toxic to many forms of insect life attacking plants and animals and those occurring in the home. Rotenone is a constituent of many plants of the *Leguminosae* family and at present the principal commercial sources are *Derris elliptica* (usually referred to merely as "derris") and *Lonchocarpus nicou* (known commercially as "cubé"), which are imported from the British and Dutch East Indies and the northern parts of South America. It has been known for many years that the roots of these plants contain ingredients highly toxic to insects and some other forms of animal life, but it is only during the past five years that their commercial use for insect control has reached large proportions. There are now scores of commercial insecticides on the market that owe their activity to these materials or extracts made from them. In 1936 this country imported more than two and one-half million pounds of derris and cubé in the form of unground and powdered roots. The importations for 1937 will greatly exceed this figure.

Another plant material that is used extensively for insecticidal purposes is pyrethrum or insect powder, which consists of the powdered flower heads of certain species of chrysanthemum. While the toxicity of pyrethrum to insects was known more than one hundred years ago, its use as an insecticide has increased tremendously in recent years. We now import approximately 13,000,000 pounds annually, the greater part of which comes from Japan. Experiments are being carried on by the United States Department of Agriculture and some of the State Experiment Stations to determine whether or not it can be grown profitably in this country.

The war against the insects has become a world-wide movement. People are beginning to realize that an adequate supply of food, both vegetable and animal, would be impossible without the use of insecti-

cides and fungicides. In this fight our Federal Government employs hundreds of scientists and trained men, who are devoting their lives to this work, and numerous field laboratories are maintained throughout the country. Every state has its corps of expert workers and investigators. Some of our large cities maintain city entomologists, and California has its county entomologists. Practically all of our agricultural colleges and universities, as well as the medical schools, are training men in insect biology; a number of our large manufacturers and oil companies, as well as endowed non-profit institutions, maintain research laboratories manned by trained experts, where extensive research on methods of control of insects and the development of new insecticides are being conducted. Among the latter organizations may be mentioned The Mellon Institute of the University of Pittsburgh. It was established in 1913 for the purpose of providing a means for a manufacturer, or association of manufacturers, to investigate new materials and processes in chemistry and allied subjects. Practically from the beginning of its foundation it has conducted, through grants from manufacturers, researches on insecticides. A similar organization, although somewhat more limited in scope, is The Crop Protection Institute, which was organized in 1920 under the auspices of the National Research Council. At that time manufacturers of insecticides were producing the standard materials, such as lead and calcium arsenates, Paris green, and lime sulfur solution, but practically none of them employed experts in the biological sciences or possessed research departments for the investigation of pest control problems. The Institute was established for the purpose of providing an agency that could make thorough and impartial tests of insecticides and fungicides for manufacturers and commercial firms that desired to improve their products or develop new ones to meet the needs of farmers for controlling crop pests.

The Boyce Thompson Institute for Plant Research, Inc., 1086 North Broadway, Yonkers, New York, officially opened in 1924, conducts "fundamental research on practical plant problems," including work on insecticides.

The National Association of Insecticide and Disinfectant Manufacturers has recently established a fellowship at the Ohio State University to study and develop laboratory methods for evaluating insecticides for use against household insects.

Probably the most extensive researches conducted by a single manufacturer for the purpose of developing new and more effective insecticides and fungicides for agricultural use are those of the Du Pont Company of Delaware. The researches of this company are directed especially toward a study of the insecticidal and fungicidal properties of synthetic organic compounds. Among other manufacturers actively engaged in research work in this field may be mentioned Röhm & Haas Co., Philadelphia, Pa.;

McCormick & Co., Baltimore, Md.; California Spray Chemical Corporation, Berkeley, Calif; American Cyanamid & Chemical Corporation, New York, N. Y.; and several of the larger oil companies and chemical manufacturers.

In addition to the agencies mentioned, there are in the United States many entomologists engaged in private practice as consultants, and in practically all of the larger cities professional so-called "exterminators" are engaged exclusively in contract work to free buildings of insect pests. In New York City alone, according to a survey made by the American Institute of Health, an army of 400 such men is continuously so employed. This number does not include employees of hotels, apartment houses, stores, restaurants, and food warehouses who are occupied full or part time in combating insect infestations.

Many laws have been passed to control the marketing of commercial insecticides. The first such law in this country was passed by the General Assembly of Louisiana in 1890, and the second by New York in 1898. Both of these laws applied only to Paris green, which at that time was the most important agricultural insecticide. As the insecticide industry increased it soon became apparent that a Federal law was needed. The numerous reports by growers of damage to crops and fruit trees through the use of improperly made material and the unsatisfactory state of the industry at that time made it imperative that some action be taken to win the confidence of the public in these articles of trade. After considerable agitation and many conferences among those interested in the subject and hearings before Congressional committees a law was introduced, which was passed in 1910 and became effective January 1, 1911. To the Association of Economic Entomologists belongs most of the credit for the procurement of this legislation, although it had the backing of the leading insecticide and fungicide manufacturers of the country and as a regulatory law stands out as unique in this respect. No regulatory law that has been passed by Congress more nearly affects the welfare of every individual of the nation.

At the time of the passage of this Act the insecticide industry was in a very unsatisfactory condition. Most manufacturers knew little regarding methods of control of insects and fungous diseases, and much of the material on the market was improperly manufactured and ineffective for the purpose for which it was recommended. Competition was such that scrupulous manufacturers were not able to meet it without lowering the quality of their products. The passage of this law marked the beginning of a great change in the insecticide industry,—its growth has been phenomenal and there is little resemblance between the insecticidal products on the market to-day and those on the market at the time of its passage. Other factors have, of course, aided in bringing about the increased use of insecticides. As a result of researches by entomologists

and others, great progress has been made in methods for the control of insects, new products have been developed, and better methods of application devised.

For the most part the trade is in sympathy with the Federal law since it tends to keep down unfair competition and to instil greater confidence in the buyer for the products subject to its provisions. Laws applying to insecticides have been passed by about one-half of the states. Most of these are similar in their requirements to the Federal statute, but few of them are being vigorously enforced due, primarily, to a lack of the necessary funds.

In addition to laws regulating the marketing of insecticides, plant quarantine laws have been enacted for the purpose of preventing the introduction of plant pests into this country and limiting their spread after they have reached here. The first law of this character enacted in this country was passed by the State Legislature of California in 1881. It was not, however, until 1912 that a national law, the Federal Plant Quarantine Law, was enacted by the United States Congress. This act regulates the importation into, as well as the movement between the states, of all plants and plant products that may carry plant diseases and insect pests. Its enforcement has been of inestimable benefit in preventing or retarding the spread of destructive insect pests in this country.

We are still behind some countries in legislation designed to control the ravages of insects. Germany, for example, enacted a law in March of this year empowering the Government to compel farmers to apply insecticides and institute other officially prescribed practical measures for combating plant pests and disease. If necessary, the Government itself will do this work and require the farmer or establishment benefited to contribute to the cost of the service.

In considering this subject of insect control it would be amiss not to make reference to the valuable work of this Association in the development and adoption of methods of analysis of commercial insecticides, which have been of incalculable aid in connection with the enforcement of regulatory laws. This work was initiated by the Association in 1898 and has been continued without interruption since that time. Dr. A. L. Winton, who was President of the Association that year, made reference in his presidential address to the growing need for accurate methods of analysis of these products and recommended "that a referee on insecticides and fungicides by appointed each year to investigate methods for the determination of the valuable constituents of these products." Affirmative action was taken on this recommendation, and Dr. de Schweinitz, then a scientist in the Bureau of Animal Industry of the Department, was appointed referee for the following year,—the first referee of the Association on this subject. As a result of the work of members of this Association and collaborators since that time many accurate and valuable methods

of analysis for these products have been developed and adopted by the Association. These methods, published in *Methods of Analysis, A.O.A.C.* and in Wiley's *Principles and Practice of Agricultural Analysis*, Vol. II, issued by this Association, constitute the most inclusive compilation of methods relating to insecticides and fungicides now in print.

The work of the Association, however, has not been completed. As pointed out by Dr. MacIntire in his Wiley's memorial address before this Association two years ago, "The marked increase in the necessary use of various insecticides has also injected new problems relating to the effects induced by their residues." One of these problems has already been referred to,—that of the health menace by reason of the residue left on the crop at harvest time. Another, which may become equally important, is the effect the accumulation of these toxic compounds in the soil may have on the growth of plants and the possible injury to man and animals from eating products grown on such soils. One of the leading fruit-growing areas in the Pacific Northwest has received as much as 7,000,000 pounds of lead arsenate annually for the past twenty years, and it has already been shown that in some sections injury to vegetation through arsenic accumulation in the soil is manifest. The amount of calcium arsenate usually recommended to be used on cotton is from 15 to 20 pounds per acre annually, which over a 25-year period would average 450 pounds per acre. For the destruction of the grubs of the Japanese bettle in the soil it is being recommended that lead arsenate be worked into the soil at the rate of 10 pounds per 1000 square feet, or 430 pounds per acre. This treatment is said to be effective against the beetle grubs for about five years, when the application must be repeated. Nurserymen located in the Japanese bettle infested area who ship outside of the quarantine region are required by Government regulations to treat the soil on which they grow their nursery stock by certain specified methods in order to destroy the beetle grubs in the soil. One of these methods is to work lead arsenate into the soil at the rate of 1500 pounds per acre. Owing to the insolubility of lead arsenate its removal from the soil by leaching is very slow, and one would expect that such heavy applications would in time seriously injure the soil for cultivation purposes. Selenium compounds are now being used to some extent on crops for the control of certain insects, particularly red spiders, and, owing to the extremely toxic character of selenium to human beings and the fact that it is taken up from the soil by the growing plant, fear has been expressed that man and animals might be injured by eating products grown on such soils. Some investigational work on the absorption of selenium by plants has already been done by the Bureau of Chemistry and Soils. The absorption of gases by fumigated food products is also a problem for the food control officials. Hydrocyanic acid is used extensively for fumigating a great variety of food products that are subject to insect infestation, such as fresh and dried fruits, vegetables, cereals

and other edible products packed in cartons, such as flour, meal, nuts and nut meats, cheese, and candies. Investigation has shown that while hydrocyanic acid is usually dissipated quite readily from many of such fumigated products, in the case of some it is held for long periods of time.

In that part of this paper relating to methods for the control of insect pests I have stressed the use of insecticides. It is not intended, however, to belittle other control measures, such as biological, through the use of insect parasites; plant selection and breeding to produce resistant varieties; and cultural practices, in which some striking developments have been made, but the problem is essentially one of applied chemistry and one in which the chemist must play a leading part.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY

WEDNESDAY—AFTERNOON SESSION

REPORT OF EDITORIAL BOARD

The sale of the 1935 Edition of *Methods of Analysis* is progressing very well. Of the 5,000 copies obtained last October, practically half has been sold. After the first year, however, the sales slow up and are approximately 60-80 copies each month. Because of the healthy condition of our finances the Executive Committee reduced the price from $5.00 to $4.00 to members. This apparently has been appreciated. Another action of the Committee provided for a discount to colleges and universities when five or more copies are ordered for student and reference use.

Perhaps it is through the popularity of our *Methods of Analysis* that more persons are becoming acquainted with our *Journal* and are subscribing. This condition is plainly shown in our receipts for subscriptions, which are approximately $500 greater this year than last year.

The condition in regard to Wiley's *Principles and Practice of Agricultural Analysis* has not changed. Dr. Browne has reported so fully in previous years in regard to the apathy of the publishers that a review of the matter is unnecessary. Nine copies were sold this year.

The action of the Executive Committee and the financial statement will be included in the report of the Secretary-Treasurer. Dr. Bailey and Mr. Lepper will give brief reports as Chairmen of the Committees on *Methods of Analysis* and *The Journal*, respectively,

W. W. SKINNER, *Chairman*

Approved.

REPORT OF EDITORIAL COMMITTEE OF *THE JOURNAL*

This year *The Journal* has repeated its success of last year, both from a financial standpoint, as the report of the Secretary-Treasurer shows, and also with respect to the number of pages published. The record volume of 679 pages of last year exceeds by only 16 pages the number this year.

In keeping with the decision of the Association to increase expenditures for publication, the availability of the additional space that could be largely allotted to contributed papers was brought to the attention of research

workers in agricultural chemical and other closely related fields. A letter was addressed to all directors of agricultural experiment stations in the United States, inviting their consideration and that of their staffs to *This Journal* as a medium of publication for papers reporting their researches. It was stressed that while papers involving development of methods were of interest, papers on other subjects of agricultural interest were equally desirable, in light of the broad purposes of the Association as expressed in the constitution. Response to the invitation has not been reflected in an increase in the size of *The Journal* this year, but it is expected that the coming year will see an increase in both number and scope of the contributed articles.

Our journal, dedicated as it is to agriculture, is regarded by those whose interests lie in agricultural chemistry and related fields as the preeminent journal, in which it is expected that reports and papers on the development of these subjects will be found. It reaches those readers as readily as do journals that perhaps enjoy a wider circulation but reach readers of much more diversified interests. The exchanges established by our Association with chemical societies and publishers of other journals have placed our journal in reference collections throughout the world. An appreciation of these facts should lead our members to use their journal as a medium of publication, thereby contributing to the increased prestige and growth that is planned for it.

The continued progress of our journal is attributed to the effective work of our Associate Editor and the appreciation of the Association is extended to Miss Marian Lapp.

H. A. Lepper

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF *METHODS OF ANALYSIS*

There has been a gratifying demand for the new edition of *Methods of Analysis*. Many favorable reviews have been received from both foreign and domestic sources. A few criticisms have been made, and these will be of value in preparing the next revision.

Of the chapters entered by name only, viz., Sewage, Agricultural Dust, Fish and Other Marine Products, Vitamins, Bacteriological Methods, and Microchemical Methods, all excepting two, Sewage and Agricultural Dust, are now being actively studied and methods should be available for the next edition.

No edition of our book of methods remains "new" for very long, and in another year it will not be too soon to begin plans for the 1940 edition.

E. M. Bailey, *Chairman*

Approved.

No report was given by the Committee on Quartz Plate Standardization and Normal Weight.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

Official, First Action

DOLOMITE

Dolomite is a mineral composed chiefly of carbonates of calcium and magnesium in substantially unimolal proportions.

PRIMARY FERTILIZER COMPONENTS

Primary Fertilizer Components are those at present generally recognized by law as necessary to be guaranteed in fertilizers, namely: nitrogen, phosphoric acid, and potash.

SECONDARY FERTILIZER COMPONENTS

Secondary Fertilizer Components are those other than the "primary fertilizer components" that are essential to the proper growth of plants and that may be needed by some soils. Some of these components are calcium, magnesium, sulfur, manganese, copper, zinc, and boron.

BAT MANURE

Bat manure is the dry excrement of bats.

BAT GUANO

Bat guano is partially decomposed bat manure.

ANALYSIS

The word *analysis*, as applied to fertilizer, shall designate the percentage composition of the product expressed in those terms that the law requires and permits.

First Reading as Tentative

CALCIUM NITRATE

Calcium nitrate (nitrate of lime) is a commercial product consisting chiefly of calcium nitrate, and it shall contain not less than fifteen per cent (15%) of nitrogen.

SUPERPHOSPHATE

Superphosphate, 24 per cent or below, is a commercial product consisting largely of available phosphates and calcium sulfate resulting from treating ground phosphate rock with sulfuric acid. The grade that shows the available phosphoric acid should always be used as a prefix to the name.

Example: 18 per cent superphosphate.

Superphosphate, over 24 per cent, is a commercial product consisting largely of available phosphates and some calcium sulfate resulting from treating ground phosphate rock with phosphoric acid or both phosphoric acid and sulfuric acid. The grade that shows the available phosphoric acid should always be used as a prefix to the name.

Example: 45 per cent superphosphate.

AMMONIATED SUPERPHOSPHATE

Ammoniated superphosphate is the product obtained when superphosphate is treated with ammonia or with a solution containing free ammonia and other forms of nitrogen dissolved therein.

L. S. Walker
G. S. Fraps
L. E. Bopst
W. H. MacIntire
W. Catesby Jones

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

The continued success of our Association in the development of analytical methods rests in large part on securing an adequate number of collaborators. It is only the exceptional referee in our Association that does not experience difficulty in securing this cooperation to the extent he feels desirable. At the present time referees are furnished the names of collaborators who have signified their interest in the subject at the time of registration at the convention, or they obtain them by personal solicitation among those whom they believe are interested. By these procedures a number of chemists who may not be attending the meetings, or who are not known to the referee, may be reached. Your Committee believes that the Association can assist, with only nominal cost, in bringing more collaborators and referees together. It is suggested that after the meeting a list of the proposed studies be circulated among potential collaborators to ascertain the subjects on which they would like to collaborate. Each referee could then be furnished the names and addresses of these supplementary collaborators. It is recommended that this proposal be tried this coming year to determine whether it will enlarge the field of activities of the Association.

Reports of the several subcommittees will be presented by the respective chairmen.

H. A. LEPPER

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By G. E. GRATTAN,† H. A. HALVORSON, and E. L. GRIFFIN

STANDARD SOLUTIONS

It is recommended—

(1) That study of direct methods for standardizing acids be continued.

(2) That standardization of iodine solution be studied.

(3) That standardization of sodium thiosulfate solution be studied.

INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

It is recommended—

(1) That the mercury reduction method submitted by the referee for the determination of pyrethrin I in pyrethrum powder and mineral oil pyrethrum extracts (see p. 78) be adopted as a tentative method and that collaborative work be continued.

(2) That the chloroform extraction method for the determination of

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* These recommendations, submitted by Subcommittee A, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1935.

† The Chairman of this committee, G. L. Bidwell, died on February 20th, 1937. G. E. Grattan succeeded to the chairmanship, and E. L. Griffin was appointed to fill the vacancy on the committee.

rotenone in derris and cube powder (see p. 79) be adopted as a tentative method and that collaborative work be continued.

(3) That the lead chlorofluoride method for the determination of fluorine be studied next year.

(4) That further study be given the determination of naphthalene in poultry lice powders.

FEEDING STUFFS

It is recommended—

(1) That an associate referee be appointed to study the determination of ash in feeding stuffs and that particular attention be given to temperature control of the ignition.

(2) That the determination of manganese be studied, and that if possible the work be correlated with one of the methods already in use.

(3) That the study of the determination of lactose be continued.

(4) That work on the determination of fluorine in feeding stuffs be discontinued until such time as a method for the same determination in foods is adopted.

(5) That further studies be made of methods for the detection of adulteration of condensed milk products.

(6) That a study be made of methods for the detection of adulteration of cod liver oil.

(7) That further study be given to the microanalytical detection of iodine in feeding stuffs.

(8) That the Elmslie-Caldwell method for the determination of iodine in mineral feeds, *This Journal*, **18**, 338 (1935), be adopted as tentative and that collaborative work be continued.

(9) That collaborative work be done on the present tentative method for the determination of calcium oxide in mineral feeds (p. 347, **44**) with a view to the final adoption of the method as official.

(10) That the qualitative tests for protein (p. 337–8, **10–17**) be made official (final action).

(11) That studies on the determination of fat in fish meal be continued.

(12) That the Hughes-Peterson method for the determination of carotene, *This Journal*, **20**, 464 (1937), be studied further.

(13) That the potassium dichromate standards be rechecked against pure beta carotene and the best conditions for accurate application be established and used as the colorimetric standards for the Hughes-Peterson procedure.

(14) That the study of the application to the carotene determination of the neutral wedge photometer and the photoelectric colorimeter be continued.

(15) That the study of various factors affecting bone ash determination and other criteria used for interpreting the amount of calcification in young chicks be continued.

(16) That the qualitative (picrate) test for the determination of glucosides in feeds and similar materials (p. 347, **45**) be adopted as official (final action).

(17) That the modified alkaline titration method and the acid titration method for hydrocyanic acid (p. 348), be further studied with a view to adopting one or the other as official.

(18) That the effects of various concentrations of sodium hydroxide in the alkaline titration method for hydrocyanic acid be studied.

(19) That the use of a single indicator solution in this method be given further study.

(20) That the vacuum oven and the electric air oven methods for the determination of moisture in wheat flour, dried and malted milk, and grain and stock feeds be further studied as a group with a view to unification.

(21) That the work of correlating the moisture methods of the Association be continued.

FERTILIZERS

It is recommended—

(1) That an associate referee be appointed to study methods for the determination of calcium and sulfur in fertilizers.

(2) That the Associate Referee on Potash be requested to ascertain whether any other state besides California forbids the use of the present official method for potash on account of provisions of the laws regarding water-soluble potash and to make any recommendations regarding this matter that seem desirable.

(3) That the Associate Referee on Phosphoric Acid continue the study (a) of the influence of different filter papers on the determination of water-soluble phosphoric acid and (b) of the effect of permitting the washed residue to stand for a time before digestion in the ammonium citrate solution, and that collaborative work be carried on.

(4) That further study be made of the nature of the citrate-insoluble components of phosphate materials with a view to improving the method of determining the availability of such materials.

(5) That the method for the determination of water-insoluble nitrogen in cyanamide (p. 27, **36**) be adopted as official (final action).

(6) That the reduced iron method for the determination of nitrate and ammoniacal nitrogen in mixed fertilizers or nitrate salts (p. 26, **31**) be deleted (first action).

(7) That the Devarda method (p. 26, **33**) be revised as suggested by the referee (see p. 77).

(8) That the Associate Referee on Nitrogen cooperate further with the Referee on Standard Solutions with a view to adopting one method of standardization for acids.

(9) That the official method for the determination of nitrate nitrogen

(p. 27, **34**) be revised as published last year, *This Journal*, **20**, 50 (1937) (final action).

(10) That the study of the use of a factor weight or weights in the determination of potash be continued.

(11) That the barium chloride method for the determination of potash, Method II (p. 31, **45, 46, 47**) be deleted (first action).

(12) That further study be made of the determination of potash by filtration after ignition and solution when platinum or silica dishes are used.

(13) That further study be made of the need for providing additional plantinum solution concentrations.

(14) That a study be made of the recovery of platinum with a view to recommending one or more of the procedures.

(15) That additional investigation be made of some modification of the official method for the determination of potash (p. 30) to prevent foaming during the boiling of the sample.

(16) That additional studies be made of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination.

(17) That the solvent action of acid alcohol on potassium chloroplatinate be studied.

(18) That further study be given to the following suggested change in the last two sentences of section **44(a)**, p. 30:

Weigh and remove the chloroplatinate precipitate by washing with hot H_2O, using slight suction. Wash with 80% alcohol three times, dry as before, and weigh (loss equals K_2PtCl_6). Calculate to K_2O.

(19) That an associate referee be appointed to study a method for the determination of copper and zinc in fertilizers and that he cooperate with the associate referee on other secondary elements to ascertain whether or not the analysis can be made on one solution.

(20) That the present tentative method for the determination of acid- and base-forming quality of fertilizer (p. 34, **55**) be modified by the substitution in the titration of 0.5 *N* sodium hydroxide solution in place of 1.0 *N* sodium hydroxide solution.

(21) That further study be given to the same method in regard to changing the indicator from methyl red to bromphenol blue and the use of a filter paper cone to prevent spattering.

(22) That in the same method the elimination of water-insoluble material coarser than 20-mesh before the method is applied be studied further.

(23) That the basicity of the phosphate rock and other factors that affect this method be studied further.

(24) That the method entitled "Total Magnesia" (p. 34, **54**) be changed to read "Acid-Soluble Magnesia" and including the changes

adopted last year, *This Journal*, **20**, 51 (1937), be made official (final action).

(25) That the method submitted by the associate referee for the determination of magnesia in water-soluble compounds used as sources of magnesium in mixed fertilizers be adopted as a tentative method.

(26) That the study of methods for the determination of active magnesia in mixed fertilizers be continued.

(27) That the method submitted by the associate referee for the determination of acid-soluble manganese be studied collaboratively and that other methods for manganese be compared.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That a study be made of the adaptability of the boiling ammonium chloride method to the evaluation of rate of availability of ground limestone and dolomites.

(2) That studies of the factors that influence the *p*H value of soils in the arid and semi-arid regions be continued.

(3) That work be continued on the determination of selenium in soils.

(4) That the studies of liming materials be continued.

(5) That since no report on fluorine or arsenic in soils was presented, these studies be continued.

PLANTS

It is recommended—

(1) That the studies of less common metals be continued.

(2) That the studies of total chlorine be continued.

(3) That the studies of carbohydrates be continued.

(4) That the studies of inulin be continued.

(5) That the Referee on the Forms of Nitrogen be retained although there seems to be no problem at the present time.

(6) That the method for the determination of hydrocyanic acid be studied further and that collaboarative work be undertaken.

(7) That the work be continued on the Hicks method, *J. Ind. Eng. Chem.*, **5**, 650 (1913), for the determination of potassium only.

(8) That in the tentative method for the determination of sodium only (p. 126, **18**) the section reading "allow to cool to 20°" be changed to read "allow to cool to about 30°," and that the method be continued as tentative.

(9) That the present tentative perchloric acid method for the determination of sodium and potassium (p. 126, **16**) be studied with a view to shortening and improving the directions for preparation of the solution.

ENZYMES

It is recommended—

(1) That work on the subject of pepsin be discontinued until such time as it can be undertaken with some similar problem.

(2) That the method of Balls, Swenson, and Stuart for the assay of papain, *This Journal*, **18**, 140 (1935), be adopted as tentative and given further study.

(3) That further study be given the method of Balls and Hoover for the clotting of milk by papain, *J. Biol. Chem.*, **121**, 737 (1937).

LIGNIN

It is recommended that further study be given to the determination of lignin in plants.

PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

It is recommended—

(1) That study of the following methods of testing varnishes be continued: Abrasion resistance, hardness, skinning, and alkali resistance, and that an associate referee be appointed.

(2) That study on the accelerated weathering of paints be continued.

VITAMINS

It is recommended—

(1) That the biological method proposed by the associate referee for the determination of vitamin B in feeding stuffs be given further consideration with a view to its adoption as a tentative method.

(2) That collaborative work be done to establish the accuracy and reliability of spectrophotometric equipment by means of a stable inorganic solution absorbing in the region of 328 $M\mu$.

(3) That the tentative method for the assay of vitamin D milk, *This Journal*, **20**, 78 (1937), be revised as indicated by the associate referee (see p. 90) and that further collaborative studies be made.

(4) That experimental and collaborative work be continued on the biological methods for the assay of vitamin D carriers.

LEATHERS AND TANNING MATERIALS

No report was presented.

It is recommended that the referee conduct studies to keep the methods up to date.

DISINFECTANTS

It is recommended that the phenol coefficient method (pp. 68–72, 141–146) be adopted as official (first action).

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES

By L. B. BROUGHTON (University of Maryland, College Park, Md.), *Chairman;* H. J. FISHER and A. E. PAUL

NAVAL STORES

It is recommended that the modifications recommended by the referee for the saponification number determination for rosin (see p. 80) be approved and that study of the subject be continued.

TURPENTINE

It is recommended that studies on the subject of turpentine be continued.

RADIOACTIVITY

No report on quantum counter was received. It is recommended that the subject be continued.

No report on gamma ray scope was submitted. It is recommended that the topic be continued.

COSMETICS

No report was received. It is recommended that the study be continued.

DRUGS

MICROCHEMICAL METHODS FOR ALKALOIDS

It is recommended that the tests proposed by the associate referee for hydrastinine, ethylmorphine (dionine), benzylmorphine (peronine), and apomorphine be adopted as tentative (see p. 91), and that the subject be continued with particular attention given to berberine, coniine, narcotine, narceine, and cytisine.

MICROCHEMICAL METHODS FOR SYNTHETICS

It is recommended that the microchemical methods submitted by the associate referee for the identification of acetylsalicylic acid, benzoic acid, and salicylic acid be adopted as tentative (see p. 93), and that sulfanilamide, mandelic acid, and diallyl barbituric acid be studied.

HYPOPHOSPHITES

It is recommended that this subject be continued with a view to applying the bromine method to the assay of sirups containing hypophosphites in known quantities.

SANTONIN, PHENOLPHTHALEIN, AND CALOMEL IN TABLETS

Because of its relative unimportance, it is recommended that this topic be closed.

DAPHNIA METHODS

The associate referee gave a most interesting verbal report of progress. It is recommended that the topic be continued.

HEXYLRESORCINOL

No report was received. It is recommended that the subject be continued.

ERGOT ALKALOIDS

It is recommended that the topic be continued.

NITROGLYCERINE IN MIXTURES

It is recommended that the subject be reassigned.

GUAIACOL

It is recommended that the subject be reassigned to the same associate referee for study with respect to guaiacol itself, and collaborative study of the method for both guaiacol and guaiacol carbonate.

BIOLOGICAL TESTING

No report was given by the associate referee. It is recommended that the topic be continued.

IODINE OINTMENT

It is recommended that as suggested by the referee and associate referee the proposed method for the determination of free iodine be tentatively adopted (see p. 94). The method should be retained in a tentative status, however, and not advanced to adoption as an official method. It is also recommended that the proposed method or other methods for combined iodine be further studied for an additional year.

ACETOPHENETIDIN IN THE PRESENCE OF CAFFEINE AND ASPIRIN

It is recommended that the topic be reassigned.

PYRIDIUM

The associate referee devised a method that has yielded excellent results in his laboratory. He has, however, not been able to submit the method to collaborative work on authentic samples. Because of his own results and the known general applicability of the method to azo dyes, it is recommended that the method be adopted as tentative (see p. 94), and that the subject be closed.

GUMS

The associate referee was unable to do any work on this subject during the year. It is recommended that it be reassigned for further study.

CINCHOPHEN IN THE PRESENCE OF SALICYLATES

It is recommended that the method proposed by the associate referee be adopted as tentative (see p. 95), and that the subject be closed.

THEOBROMINE AND THEOBROMINE CALCIUM TABLETS

It is recommended that next year the present tentative method and the new method proposed by the associate referee be studied together on samples of known composition in order to reach a conclusion as to whether the change proposed is warranted.

CHLORBUTANOL

It is recommended that the topic be continued because the collaborative results were not completely satisfactory.

ASPIRIN AND PHENOLPHTHALEIN MIXTURES

It is recommended that this subject be continued.

HOMATROPINE IN TABLETS

It is recommended that the method proposed by the associate referee and subjected to collaborative study be adopted as tentative (see p. 95), and that the subject be closed.

CUBEBS

The proposed distillation method is included in *Methods of Analysis* under spices and is therefore available for *unofficial* use on any other similar product, such as cubebs. The National Formulary includes an extraction method for cubebs and the requirements for volatile oil in the National Formulary are based on determinations made by that method. Under the circumstances it is not desired to adopt the associate referee's method at this time. It is suggested that the method be submitted to the N. F. Revision Committee for its consideration in connection with the minimum requirement for volatile oil, since the results obtained by the two methods may not fully agree.

AMINOPYRINE AND PHENOBARBITAL IN MIXTURES

It is recommended that further study be given to this topic.

EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE

It is recommended that the methods proposed by the associate referee for the determination of caffeine and of potassium bromide in effervescent potassium bromide with caffeine be adopted as tentative (see p. 96), and that the subject be closed.

ELIXIR OF TERPIN HYDRATE AND CODEINE

It is recommended that this subject be continued next year because the work planned by the associate referee is not entirely complete.

EMULSIONS OF COD LIVER OIL

It is recommended that the title of this project be changed to "Emulsions" so that it may include all medicinal emulsions, and that the work be continued.

OINTMENT OF MERCURIC NITRATE (CITRINE OINTMENT)

It is recommended that this subject be reassigned and that the referee's suggestions be given consideration along with any other modifications that may increase the accuracy of the method.

RHUBARB AND RHAPONTICUM

It is recommended that this subject be further studied.

THEOPHYLLINE SODIUM SALICYLATE

It is recommended that this subject be reassigned under the title "Theophylline," with the intention of attempting to replace the present

tentative method by one of the two proposed methods of the associate referee. A statement should be included in regard to the mixtures for which the methods are suitable.

NEW SUBJECTS

It is recommended that the following new topics be assigned to associate referees for study during the coming year: Sulfanilamide and mandelic acid.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By W. B. WHITE (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*, J. O. CLARKE, and G. G. FRARY

CANNED FOODS

It is recommended—

(1) That the method presented by the referee for the determination of alcohol-insoluble material in canned peas (see p. 89) be adopted as official (first action).

(2) That the method for the determination of chlorides in tomato juice, *This Journal*, **20**, 78 (1937), be adopted as official (first action).

(3) That studies of methods for the analysis of tomato juice be continued.

(4) That studies of methods for quality factors and for fill of container be continued.

DAIRY PRODUCTS

It is recommended—

(1) That studies of methods for the detection of neutralizers in dairy products be continued, and that particular attention be given to the ratio between titratable acidity and lactic acid.

(2) That the method for fat in malted milk (p. 282) and especially the precautions suggested by the referee be further studied with a view to obtaining closer agreement of Reichert-Meissl values.

(3) That methods for the determination of lactic acid in dried milk be further studied.

(4) That the associate referee study methods of isolating fat from cheese for the determination of fat properties and constants, giving special attention to such treatments as promise a minimum change in the properties of the fat and keeping in mind the applicability of such methods to other dairy products.

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* These recommendations, submitted by Subcommittee C, were approved by the Association. Unless given otherwise, all references are to *Methods of Analysis, A.O.A.C.*, 1935.

(5) That studies of methods for the determination of casein in malted milk be continued.

(6) That the official methods for preparation of sample (p. 288) and the official indirect method for the determination of fat in butter (p. 289) be clarified as recommended by the referee (see p. 84).

(7) That the tentative method for determination of ash in cheese (p. 291) be adopted as official (final action).

(8) That the tentative method for determination of total chlorides in cheese, p. 291, **93**; *This Journal*, **20**, 70 (1937), be adopted as official (final action).

(9) That the method for determining the degree of pasteurization of milk and cream submitted by the referee (see p. 82) be adopted as tentative and submitted to further study.

(10) That the tentative method for the determination of specific gravity of milk (p. 264, **3**) be changed as suggested by the referee (see p. 84).

(11) That the method submitted by the referee for approximating the total solids in milk be adopted as tentative (see p. 84).

(12) That studies of mounting media in the microscopic method for the identification of malted milk be continued.

(13) That studies be made of alternative methods for detecting gelatin in milk and cream.

(14) That the methods for the determination of casein (p. 265) be studied with a view to the adoption of not more than one method.

(15) That the tentative method for the determination of citric acid in milk (p. 264) be further studied.

(16) That the revision of the official optical method for the determination of lactose in milk proposed by the associate referee in 1936, *This Journal*, **20**, 70 (1937), be adopted as official (final action).

(17) That studies on other methods of clarification of milk for the optical determination of lactose be continued.

(18) That methods for the detection of decomposition in dairy products be studied.

(19) That studies of methods for the detection and determination of extraneous matter in dairy products be continued.

(20) That studies be made of methods for distinguishing between products made from cow's milk and those made from the milk of other animals.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the associate refereee define more clearly the details for determining water-soluble nitrogen and crude albumin in dried eggs, and subject these methods to further collaborative study.

(2) That work on chemical methods for detecting decomposition in eggs be continued.

(3) That studies of methods for the determination of cholesterol and fat be continued.

(4) That studies of methods for the determination of sugar, added salt, and glycerol be continued.

(5) That the rapid method submitted by the associate referee for determining the acidity of ether extract be adopted as tentative.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the methods proposed by the associate referee for preparation of sample, and the determination of ash, salt, and total nitrogen (see p. 85) be adopted as tentative.

(2) That further study be made of methods for the determination of ether extract and total solids.

GUMS IN FOODS

It is recommended that the tentative method for the detection of gums in cheese (p. 295, 106) be further studied in relation to its applicability to foods other than cheese, and that other methods for the detection of gums be studied.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That studies of methods for the detection of nitrate and nitrite nitrogen in meat products, including meat extracts and curing solutions, be continued.

(2) That studies of methods for the detection of dried skim milk in meat products be continued.

METALS IN FOODS

It is recommended—

(1) That studies be continued on methods of sample preparation of those products wherein the arsenic is tenaciously held.

(2) That the iodine titration, gold or silver sol, and the molybdenum blue colorimetric methods for the determination of arsenic be further studied as possible substitutes for the Gutzeit method.

(3) That the studies of methods for the determination of antimony and for arsenic be combined under a single associate refereeship, and that special attention be given to the separation of micro quantities of arsenic and antimony occurring simultaneously in organic or biological material.

(4) That studies on micro methods for the determination of copper be continued.

(5) That collaborative studies on methods for the determination of fluorine in phosphates and baking powder be continued, and that special attention be given to methods of sample preparation for organic materials.

(6) That the colorimetric dithizone and the electrolytic methods for the rapid determination of lead on apples and pears (p. 391, **30–33**) be made official (first action).

(7) That studies of methods for the determination of lead be continued, with special reference to oils and baking powders, and to the simplification of methods for removing interfering substances.

(8) That studies on methods for the determination of mercury be continued.

(9) That studies on methods for the determination of selenium be continued.

(10) That studies on micro methods for the determination of zinc be continued.

(11) That methods be studied for determining fumigation residues in food products.

OILS, FATS, AND WAXES

It is recommended—

(1) That the Fitelson method for the detection and estimation of tea seed oil, *This Journal*, **19**, 496 (1936), be made official (final action).

(2) That the modified Kaufmann method submitted by the associate referee for the determination of the thiocyanogen number for fats and oils (see p. 87) be adopted as tentative.

(3) That the official method for the determination of free fatty acids (p. 417) be dropped (first action).

(4) That the N.C.P.A. methods for the determination of free fatty acids in crude and in refined oils (see p. 88) be both made official (first action).

(5) That studies on methods for the determination of acetyl value and hydroxyl value be discontinued for the present.

(6) That studies be made on the application of the associate referee's method for the determination of oil in flaxseed to other commercially important oil seeds.

(7) That studies be undertaken on the Polenski method.

SPICES AND CONDIMENTS

It is recommended—

(1) That the referee's method for the assay of marjoram be studied in regard to its application to other spices.

(2) That the official methods for the determination of ash in vinegar, (p. 456, **58**) be dropped (first action).

(3) That the method proposed by the associate referee for the determination of ash in vinegar (see p. 89) be adopted as official (first action).

(4) That the Referee on Vinegar study methods for the determination of total phosphoric acid.

(5) That the official method for the determination of solids in vinegar

be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

(6) That methods for the detection of caramel in vinegar be studied.

MICROBIOLOGICAL METHODS

It is recommended that studies be continued on the microbiological examination of canned vegetables, canned tomatoes and fruits, canned fishery products, canned meats, and sugar; and that similar studies be undertaken on eggs and egg products.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By J. A. LeClerc (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman;* J. W. Sale and W. C. Jones

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the study of the lead precipitate be discontinued for the present.

(2) That the official method of Wein for the determination of maltose (p. 484, 54–55) be dropped (first action).

(3) That the work on methods for determining acetyl-methyl carbinol and diacetyl in food products be continued.

(4) That the work on methods for determining the so-called unfermentable sugars of molasses be continued.

(5) That the study of maple flavor concentrates and imitations be continued.

(6) That the study of the effect of clarifying agents upon the polarization of jellies and other pectin-containing materials be continued, but that this referee work be conducted under the subject of Fruit and Fruit Products.

(7) That studies on the determination of moisture in honey be continued.

(8) That the work on refractive indices of invert sugar solutions and the change in refractive indices with change of temperature in such products as invert sugar solutions, table sirups, etc., be continued.

(9) That the study of polariscopic methods be continued along the lines covered by the recommendations made and approved in 1931, 1932, and 1933.

(10) That study of chemical methods for reducing sugars be continued.

(11) That study of drying, densimetric, and refractometric methods be continued.

(12) That the vacuum drying method adopted by the International

* These recommendations, submitted by Subcommittee D, were approved by the Association. Unless given otherwise, all references are to *Methods of Analysis, A.O.A.C.*, 1935.

Commission for Uniform Methods of Sugar Analysis be made official (first action).*

(13) That the International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936, be adopted as official (first action).*

(14) That the International Temperature Correction Table, 1936, be adopted as official (first action).*

WATERS, BRINE, AND SALT

It is recommended—

(1) That the statement on page 506, **14(c)**, "0.0001 mg of N as NO_2," be changed to read: "0.0001 mg of N" (first action).

(2) That the determination of boron in waters be further studied.

(3) That collaborative work be conducted on the procedure presented by the Associate Referee on Mineral Salts and Effervescent Salts for the determination of moisture in these salts.

ALCOHOLIC BEVERAGES

It is recommended—

(1) That section **44(e)**, p. 158, of the method for the determination of extract in malt be rewritten (see p. 81).

(2) That the pressure method described by the associate referee for determining the CO_2 in beer be compared with the present tentative method (p. 151, **19**).

(3) That the tentative methods for the following determinations in beer (chap. **XX**) be further studied: Extract in original wort, real degree of fermentation, total acid, reducing sugars, dextrin, direct polarization, SO_2, pasteurization, chloride, preservatives, and hydrogen-ion concentration.

(4) That methods for the determination of heavy metals (Fe, Cu, Pb), As, and fluorine be studied.

(5) That the viscometric method outlined by the associate referee for the determination of the proteolytic activity of malt, and the edestin titration method be further studied.

(6) That the vacuum method for the determination of moisture in flour (p. 206, **2**) be studied as to its applicability to the determination of moisture in malt adjuncts (p. 161, **53**).

(7) That a special study be made of methods for the determination of fat that will be applicable to corn grits and brewers' rice and flakes.

(8) That a study of the method for determining the extract in malt adjuncts be made and that consideration be given to the suggestion to use a portion of the malt in the boiling operations.

(9) That work on the determination of alcohol in wines by the use of the ebullioscope be discontinued.

(10) That the study of new methods of analysis to detect adulteration of distilled spirits be continued.

* Approved by a three-fourths vote of the Association.

(11) That collaborative work on the determination of sulfur dioxide in wines and beers be conducted.

(12) That collaborative work on the tentative methods for the determination of volatile esters, gamma-undecalactone, and benzaldehyde in cordials and liqueurs be conducted.

(13) That the study of the saponification of esters in wines by lead acetate be continued.

(14) That the determination of volatile acidity in wine be further studied.

(15) That special study be made of the diastatic activity of malt.

(16) That special study be made of aldehydes in whiskey and other potable spirits, volatile acids in distilled spirits, and wood alcohol in brandy.

FOOD PRESERVATIVES

It is recommended—

(1) That the method for the determination of saccharin in non-alcoholic beverages (p. 435, **15**) be further investigated and if found satisfactory, submitted to collaborative study.

(2) That the work on the Illing method for determination of benzoate of soda, *Analyst*, **57**, 224 (1932), which was found to be suitable for sausage, be continued with respect to its suitability for other food products.

(3) That further studies based on the work of Tortelli and Piazza, be made on the qualitative test for saccharin (p. 434, **13**).

COLORING MATTERS IN FOODS

It is recommended—

(1) That collaborative work be continued in estimating ponceau SX and ponceau 3R.

(2) That investigational work be continued on the quantitative estimation of sunset yellow FCF in the presence of tartrazine.

(3) That investigational work be continued in separating and estimating quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That work on the determination of soluble solids and effect of acids on sugar on drying, electrometric titration of acids, and levo and inactive malic acid be continued.

(2) That a study of the determination of isocitric acid be conducted.

(3) That the application to fruits and fruit products of the colorimetric method for lactic acid in dried milk be studied, *This Journal*, **20**, 605 (1937).

(4) That an associate referee be appointed to study the determination of P_2O_5 in fruits and fruit products, especially jams, jellies, and preserves.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That work on the determination of glycerol, vanillin, and coumarin in imitation vanilla be conducted.

(2) That organic solvents in flavors be studied, and an associate referee be appointed for this work.

CACAO PRODUCTS

It is recommended—

(1) That collaborative work be done on the method for the determination of pectic acid in chocolate as an index of shell content.

(2) That methods for the determination of lecithin and other products used for similar purposes be studied.

BAKING POWDERS AND BAKING CHEMICALS

It is recommended that collaborative work on the Hartmann methods for the determination of tartaric acid and tartaric radical, *This Journal*, **13**, 385 (1930), be continued.

CEREAL FOODS

It is recommended—

(1) That the tentative method for the preparation of sample of bread (p. 221, **50**) be made official (first action).

(2) That the method for collection and preparation of sample of macaroni products, (p. 228, **68**) be made official (first action).

(3) That the tentative magesium acetate method for ashing cereal products, *This Journal*, **20**, 69 (1937), be made official (first action).

(4) That the statement found in **38(c)**, p. 216, *viz.*, "1 cc of the last soln = 0.0001 mg of N as nitrite," be changed to read, "1 cc of this nitrite soln = 0.0001 mg of N."

(5) That the study of methods for the determination of catalase in flour be discontinued.

(6) That work on the modified tentative method for determining starch (p. 213, **31**) be discontinued and in its application this method be limited to cereal flours.

(7) That an associate referee be appointed to study methods for the determination of sugar in flour.

(8) That an associate referee be appointed to resume the study of methods for the determination of acidity in flour.

(9) That an associate referee be appointed to initiate work for the development of an experimental baking test for soft wheat flour.

(10) That the methods for the measurement of the proteolytic activity of flour be further studied.

(11) That further study be made of the methods of extraction and determination of alkaloids and other constituents of ergot as a means of determining ergot in rye flour.

(12) That further study be made of the methods for the determination of salt-free ash in macaroni and baked products.

(13) That further study be made of methods for the determination of soya flour in foods.

(14) That the associate referee continue the study of methods for the identification of the nature of the raw material used in the manufacture of macaroni.

(15) That the associate referee continue his studies on whole wheat flour.

(16) That the associate referee continue the study of phosphated flour.

(17) That the methods for the determination of chlorine bleach, *This Journal*, **18**, 497 (1935), be further studied collaboratively.

(18) That the method for the detection of benzoyl peroxide bleach be further studied.

(19) That collaborative work be done on the measurement of total carotinoid pigments in flour (bleached and unbleached) with the neutral wedge photometer.

(20) That the method for the determination of H-ion concentration of flour be further studied.

(21) That further study be made of methods for the determination of starch in both cooked and uncooked cereal foods.

(22) That the citric acid method for the determination of milk solids in bread (p. 244, **55**) be further studied and that a blank determination be included.

(23) That the lactose method for the determination of milk solids in bread, *Cereal Chem.*, **13**, 541 (1936), be further studied.

(24) That the study of the methods applicable to the determination of cold water-soluble extract be continued and that the suggestions of the associate referee be followed.

(25) That the method for the determination of CO_2 in self-rising flour be studied collaboratively.

(26) That the suggestion made last year regarding the sterol content of cereals, *This Journal*, **20**, 66 (1937), be repeated.

(27) That studies on the color of flour be continued.

MICROCHEMICAL METHODS

It is recommended that the referee subject to collaborative study methods for which there appears to be need.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FIFTY-THIRD ANNUAL MEETING, NOVEMBER 1, 2, AND 3, 1937*

I. SOILS

No additions, deletions, or other changes.

* Compiled by Marian E. Lapp, Associate Editor. Unless otherwise given, all references in this report are to *Methods of Analysis, A.O.A.C.*, 1935, and the methods are edited to conform to the style used in that publication.

II. FERTILIZERS

(1) The method for the determination of water-insoluble nitrogen in cyanamide (p. 27) was adopted as official (final action).

(2) The reduced iron method for the determination of nitrate and ammoniacal nitrogen in mixed fertilizers or nitrate salts (p. 26) was deleted (first action).

(3) The Devarda method for the determination of nitrate and ammoniacal nitrogen (p. 26) was amended (first action) by the addition of the following sentence:

In the analysis of nitrate salts proceed as directed above but use 25 cc of the nitrate soln equivalent to 0.50 g of the sample.

(4) The first part of the first sentence of section **34(b)** of the official method for the determination of nitrate nitrogen (p. 27) was amended as published in *This Journal*, **20**, 67 (1937), (final action).

(5) The barium chloride method for the determination of potash (p. 31, *Method II*, **45**, **46**, **47**) was deleted (first action).

(6) The tentative method for the determination of acid- and base-forming quality of fertilizer (p. 34) was modified by the substitution in the titration of 0.5 *N* NaOH soln in place of 1.0 *N* NaOH soln.

(7) The name of the method entitled "Total Magnesia" (p. 34) was changed to read "Acid-Soluble Magnesia," and with the changes adopted last year, *This Journal*, **20**, 67 (1937), the method was adopted as official (final action).

(8) The following method for the determination of magnesia in water-soluble compounds used as sources of magnesium in mixed fertilizers was adopted as tentative:

MAGNESIA IN WATER-SOLUBLE COMPOUNDS

(Applicable to sulfate of potash magnesia, sulfate of magnesia, and kieserite)

Weigh a 1 g sample into a 250 cc volumetric flask, add 200 cc of H_2O, and boil for 30 min.; cool, and dilute to volume with H_2O. Transfer an aliquot to a beaker, add 2 cc of HCl, and neutralize to methyl red with NH_4OH. Bring to a boil and add, while still hot, saturated NH_4 oxalate soln dropwise as long as any precipitate is produced and then an excess sufficient to convert the Mg salts also into oxalate. Boil until the precipitate is coarsely granular. Cool, again neutralize to methyl red with NH_4OH, and allow to stand at least 4 hours. Filter, and wash with H_2O until the filtrate is free from oxalates. Proceed with the precipitation and ignition of the magnesium as directed in *Methods of Analysis, A.O.A.C.*, 1935, p. 124, **12**.

(9) Correction: In line 2 of **55(b)**, p. 34, "$Na_2CO_3 \cdot H_2O$" was changed to read "$Na_2CO_3 \cdot 10H_2O$."

III. SEWAGE*

IV. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

* Subjects for future study.

V. AGRICULTURAL DUST*

VI. INSECTICIDES, FUNGICIDES. AND CAUSTIC POISONS

(1) The following mercury reduction method for the determination of pyrethrin I in pyrethrum powder and mineral oil pyrethrum extracts was adopted as tentative:

PYRETHRIN I IN PYRETHRUM POWDER AND MINERAL OIL PYRETHRUM EXTRACTS

Mercury Reduction Method

REAGENTS

(a) *Denigé's soln.*—Mix 5 g of yellow HgO with 40 cc of H_2O, and while stirring slowly add 20 cc of H_2SO_4; add another 40 cc portion of H_2O and stir until the HgO is dissolved.

(b) *Iodine monochloride soln.*—Dissolve 10 g of KI and 6.44 g of KIO_3 in 75 cc of H_2O in a glass-stoppered bottle; add 75 cc of HCl and 5 cc of $CHCl_3$ and adjust to a faint iodine color (in the $CHCl_3$) by adding dilute KI or KIO_3 soln.

(c) *Standard potassium iodate soln.*—0.01 *M*. Dissolve 2.14 g of KIO_3, previously dried at 105°, in H_2O and dilute to 1 liter. 1 cc of this solution = 0.0044 g of pyrethrin I.

DETERMINATION

Use a quantity of sample that will contain from 20 to 75 mg of pyrethrin I.

Pyrethrum powder.—Extract the powder in a Soxhlet apparatus for 7 hours with petroleum ether, transfer to a 300 cc Erlenmeyer flask, and evaporate the ether on a water bath, heating no longer than necessary to remove all the solvent.

Pyrethrum extracts in mineral oil.—Weigh or measure the extract directly into 300 cc Erlenmeyer flasks.

Add 15 cc of 0.5 *N* alcoholic NaOH soln to the flask containing the pyrethrum extract, connect to a reflux condenser, and heat on a steam bath or electric hot plate 1–1.5 hours. Transfer the mixture to a 600 cc beaker and add sufficient H_2O to make the aqueous layer to 200 cc. Add a few glass beads, or preferably use a boiling tube, and boil the aqueous layer down to 150 cc. Transfer the aqueous layer to a 250 cc volumetric flask, add 1 g of filter-cel and 10 cc of a 10% $BaCl_2$ soln, make to volume, and allow to settle, adding more $BaCl_2$ if needed to obtain a clear soln. Filter off 200 cc, add 5 cc of H_2SO_4 (1+4), filter into a 500 cc separatory funnel, and extract with two 50 cc portions of petroleum ether. Wash the extracts with several 10 cc portions of H_2O, discard the washings, and filter the petroleum ether extract thru a plug of cotton into a clean 250 cc separatory funnel. Wash the cotton with 5 cc of petroleum ether. Extract the petroleum ether with 5 cc of 0.1 *N* NaOH, shaking vigorously. Draw off the aqueous layer into a 100 cc beaker, wash the petroleum ether with 5 cc of H_2O, and add the washings to the beaker. Add 10 cc of the Denigé reagent to the beaker and allow to stand 1 hour. Add 20 cc of acetone to the beaker and precipitate the reduced mercury with 3 cc of saturated NaCl soln. Warm the mixture to about 60°, filter thru a small filter paper, and wash with 10 cc of hot acetone, transferring all the precipitate to the filter paper. Wash with two 10 cc portions of hot $CHCl_3$, and place the filter paper and contents in a 250 cc glass-stoppered Erlenmeyer flask. Add to the flask 30 cc of concentrated HCl and 20 cc of H_2O, cool, add 6 cc of $CHCl_3$ or CCl_4 and 1 cc of ICl soln and titrate with the iodate soln. From the number of cc of standard iodate soln used in the titration calculate the percentage of pyrethrin I in the sample.

Potassium iodate reacts with reduced Hg to form mercuric Hg and I. Further addition of iodate in the presence of HCl oxidizes the I to ICl.

1. $5\ HgCl + 6\ HCl + KIO_3 = I + 5\ HgCl_2 + KCl + 3\ H_2O$;
2. $2\ I_2 + KIO_3 + 6\ HCl = 5\ ICl + KCl + 3\ H_2O$

   Combining 1 and 2 gives the following equation:
3. $4\ HgCl + KIO_3 + 6\ HCl = 4\ HgCl_2 + ICl + KCl + 3\ H_2O$.

Addition of ICl does not change the volume relations between reduced Hg and the iodate soln and aids in determining the end point in the titration of small quantities of Hg. The end point is taken when the red color disappears from the $CHCl_3$ layer. The end point is not permanent. Therefore the titration should be completed rapidly with vigorous shaking after each addition of iodate.

(2) The following method for the determination of rotenone in derris and cube powder was adopted as tentative:

ROTENONE IN DERRIS AND CUBE POWDER

Chloroform Extraction Method

Weigh 30 g of the finely powdered root into a 500 cc glass-stoppered Erlenmeyer flask. Add 300 cc of $CHCl_3$ measured at a definite room temp. Place the flask on a shaking machine and fasten the stoppers securely. Agitate vigorously for not less than 4 hours, during which period it is preferable to interrupt the shaking and allow the flask to stand at rest overnight. (As an alternative procedure the flask may be shaken continuously overnight.) Remove the flask from the machine and allow the contents to cool thoroly in a refrigerator for at least an hour. Filter rapidly into a suitable flask, using a fluted paper without suction and keeping the funnel covered with a watch-glass to avoid loss from evaporation. Stopper the flask and adjust the temp. of the filtrate to the temp. of the original $CHCl_3$.

Transfer exactly 200 cc of the mixture to a 500 cc Pyrex Erlenmeyer flask and distil until only about 25 cc of the soln remains in the flask. Transfer the extract to a 125 cc Erlenmeyer flask, using CCl_4 to rinse out the 500 cc flask. Evaporate almost to dryness on the steam bath in a current of air. Then completely remove the remainder of the solvent under reduced pressure, heating cautiously on the steam bath when necessary to hasten the evaporation. (The suction may be applied directly to the flask.) Dissolve the extract in 15 cc of hot CCl_4 and again in a similar manner completely remove all the solvent. Repeat with another 10–15 cc portion of hot CCl_4. (This treatment removes all $CHCl_3$ from the resins, and the $CHCl_3$ extract is usually completely soluble in CCl_4.) If small quantities of insoluble material are present, the purification procedure described later will eliminate them. However, if a large quantity of insoluble residue remains when the extract is dissolved in the first portion of CCl_4, filter it off and thoroly wash with further portions of hot solvent, after which treat the filtered soln plus washings as directed previously for the removal of $CHCl_3$.

Add exactly 25 cc of CCl_4 and heat gently to completely dissolve the extract. Cool the flask in an ice bath for several minutes and seed with a few crystals of rotenone-CCl_4 solvate, if necessary. Stopper the flask and swirl until crystallization is quite apparent. If at this stage only a small quantity of crystalline material separates, add an accurately weighed quantity of pure rotenone, so that the final result, expressed as pure rotenone, is at least 1 g. Then warm to effect complete soln, and again induce crystallization. At the same time prepare a saturated solution of rotenone in CCl_4 for washing. Place the flasks containing the extract and the washing solution in an ice bath capable of maintaining a temp. of 0° and allow to remain overnight.

After the extract has remained 17–18 hours in the ice bath, rapidly filter it thru a weighed Gooch crucible fitted with a disc of filter paper, removing the flask from the ice bath only long enough to pour each fraction of extract into the crucible.

Rinse the residue of crystalline material from the flask and wash under suction with sufficient of the ice-cold saturated soln (usually 10–12 cc) to remove the excess mother liquor. Allow the crucible to remain under suction for about 5 min. and then dry to constant weight at 40°, which usually requires about an hour. The weight obtained is the crude rotenone-CCl_4 solvate.

Break up the contents of the crucible with a spatula, mix thoroly, and weigh 1 g into a 50 cc Erlenmeyer flask. Add 10 cc of alcohol that has previously been saturated with rotenone at room temp., swirl the flask for a few minutes, stopper tightly, and set aside for at least 4 hours, preferably overnight, at the same temp. Filter thru a weighed Gooch crucible fitted with a disc of filter paper. Rinse the crystals from the flask and wash under suction with a soln of alcohol saturated with rotenone at the temp. of recrystallization. About 10 cc will usually be required for this procedure. Allow the crucible to remain under suction for 3–5 min. and then dry at 105° to constant weight, which should be complete in 1 hour.

Multiply the weight expressed in grams by the weight of the crude rotenone-carbon tetrachloride solvate and to the product add 0.07 g, which represents the correction for rotenone held in soln in the 25 cc of CCl_4 used in crystallization. If any pure rotenone has been added, subtract its weight from the value obtained. This gives the weight of pure rotenone contained in the aliquot of the extract representing 20 g of the sample.

(3) The phenol coefficient method (p. 68) was adopted as official (first action).

VII. CAUSTIC POISONS

No additions, deletions, or other changes.

VIII. NAVAL STORES

In the tentative method for the determination of the saponification number of rosin (p. 75), No. 30 denatured alcohol was made an alternative solvent with neutral 95% alcohol, and provided for in the second line after the figure **22**, by the addition of the words, "or 50 cc of Bur. Int. Rev. Formula No. 30 denatured alcohol (1 vol. of methanol + 10 vols. of 95% ethyl alcohol) and 20 cc of alcoholic KOH made up with No. 30 denatured alcohol."

IX. PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

No additions, deletions, or other changes.

X. LEATHERS

No additions, deletions, or other changes.

XI. TANNING MATERIALS

No additions, deletions, or other changes.

XII. PLANTS

The section reading "allow to cool to 20°," in the tentative method for the determination of sodium only (p. 126, **18**) was changed to read, "allow to cool to about 30°."

XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

XIV. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

(1) The tentative method for the determination of extract in malt, p. 158, **44(e)**, was changed to read as follows:

Extract.—Ascertain the extract yield of the wort by reference to the sp. gr. values given in Table 3, **XLII**, and calculate the extract yield of the malt by the following formula:

$$\text{Extract "as is"} = \frac{P(800+M)}{100-P}, \text{ in which}$$

P = the extract in 100 g of wort (Plato, Table 3); and
M = % H_2O in the malt.

$$\text{Extract "dry basis"} = \frac{E \times 100}{100-M}, \text{ where}$$

E = extract "as is"; and
M = % H_2O in the malt.
Report extract "as is" or on "dry basis" only to the first decimal place.

XV. WINES

No additions, deletions, or other changes.

XVI. DISTILLED LIQUORS

No additions, deletions, or other changes.

XVII. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

XVIII. COFFEE AND TEA

No additions, deletions, or other changes.

XIX. CACAO PRODUCTS

No additions, deletions, or other changes.

XX. CEREAL FOODS

(1) The tentative method for preparation of sample of bread (p. 221, **50**) was adopted as official (first action).

(2) The tentative method for collection and preparation of sample of macaroni products (p. 228, **68**) was adopted as official (first action).

(3) The tentative magnesium acetate method for ashing cereal products, *This Journal*, **20**, 69 (1937), was adopted as official (first action).

(4) In **38(c)**, p. 216, the statement, "1 cc of the last soln = 0.0001 mg of N as nitrite," was changed to read, "1 cc of this nitrite soln = 0.0001 mg of N."

(5) The tentative method for the determination of starch in flour (p. 213, **31**) was limited in its application to cereal flours.

(6) In **54**, p. 223, line 4 of the paragraph beginning, "Weigh duplicate samples," "(1+1)" was changed to "(1+4)."

XXI. COLORING MATTERS IN FOODS

No additions, deletions, or other changes.

XXII. DAIRY PRODUCTS

(1) The method for the determination of ash in cheese (p. 291, **93**) was adopted as official (final action).

(2) The method for the determination of total chlorides in cheese (p. 291, **94**), as modified by the associate referee last year, *This Journal*, **20**, 70 (1937), was adopted as official (final action).

(3) The following method, submitted by the associate referee for the determination of degree of pasteurization of milk and cream, was adopted as tentative:

PHOSPHATASE TEST FOR PASTEURIZATION[1]

COLLECTION OF SAMPLE

Proceed as directed under **1**, *Methods of Analysis, A.O.A.C.*, 1935, 264, except that no preservative shall be present and not more than 48 hours shall have elapsed between the time of sampling and receipt at the laboratory. If samples are refrigerated, observe precautions to prevent freezing.

REAGENTS

(a) *Buffer substrate.*—Dissolve 1.09 g of disodium phenyl phosphate and 11.54 g of sodium veronal in H_2O saturated with $CHCl_3$ and dilute to 1 liter. Add 10 cc of $CHCl_3$ per liter and store the reagent in a refrigerator.

(b) *Folin-Ciocalteu phenol reagent.*—Dissolve 100 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), according to Dr. Folin, and 25 g of sodium molybdate ($Na_2MO_4 \cdot 2H_2O$) in 700 cc of H_2O in a 1500 cc flask connected by a ground-glass joint to a reflux condenser. Add 50 cc of 85% phosphoric acid and 100 cc of HCl and reflux gently for 10 hours. Cool, add 150 g of lithium sulfate, 50 cc of H_2O, and 4–6 drops of liquid Br. Boil the mixture without a condenser for 15 min. to remove excess Br. Cool, transfer to a 1 liter flask, dilute to volume with H_2O, and filter. (The finished reagent should have a golden yellow color; reject it if it has a greenish tint.) Keep the reagent in the refrigerator, protected from dust. For use dilute 1 volume of this stock reagent with 2 volumes of H_2O.

(c) *Sodium carbonate soln.*—Prepare a 14% or 1.3 *M* soln of anhydrous Na_2CO_3.

(d) *Filter paper.*—Whatman No. 40, 11 cm. diam (Use only this grade as interfering substances are at times extracted from other grades.)

PERMANENT PHENOL STANDARDS

(a) *Color soln, grey.*—Dissolve in distilled H_2O, 31.9 g of Co chloride ($CoCl_2 \cdot 6H_2O$), 67.5 g of Cu sulfate ($CuSO_4 \cdot 5H_2O$), and 75 g of Ni sulfate ($NiSO_4 \cdot 6H_2O$). Add 32 cc of HCl and 45 cc of H_2SO_4 and dilute to 500 cc.

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[1] Kay and Graham, *J. Dairy Research*, **6**, 191–203 (1935); Gilcreas and Davis, 25th Ann. Rpt. Intern. Assoc. Milk Sanitarians, 1936, 15–39; 11th Ann. Rpt. New York State Assoc. Dairy and Milk Inspectors, 1937 (in press).

(b) *Color soln, red.*—Dissolve 476 g of Co chloride in distilled H_2O and filter. To the filtrate add 100 cc of HCl and dilute to 1 liter.

(c) *Color soln, blue.*—Dissolve 300 g of Cu sulfate in distilled H_2O, add 20 cc of H_2SO_4, and dilute to 1 liter. (Should crystals appear when the soln is cooled to below 20°, warm slightly before using to insure complete solubility.)

Prepare permanent color standards equivalent to phenol concentrations of from 0.01 to 0.15 mg per 0.5 cc of sample by combining the quantities of color solns **a**, **b**, and **c** indicated in the table and diluting to 10 cc with distilled H_2O in each case; *e.g.*, 0.3 cc of soln **a**+0.106 cc of soln **b**+0.96 cc of soln **c**+H_2O to make a volume of 10 cc is equivalent to a phenol concentration of 0.01 mg in 0.5 cc of sample.

Preparation of permanent phenol standards

PHENOL	COLOR SOLUTION		
	GREY (a)	RED (b)	BLUE (c)
mg/0.5 cc	*cc*	*cc*	*cc*
0.01	0.30	0.106	0.96
0.02	0.40	0.140	1.16
0.03	0.55	0.180	1.65
0.04	0.65	0.216	2.10
0.06	0.92	0.286	3.00
0.09	1 30	0.326	4.40
0.12	1.70	0.360	5.70
0.15	2.50	0.396	7.10

These color standards are suitable for use only in natural daylight. If, however, a turquoise blue, unglazed, opaque glass plate is used to deflect the light from a daylight lamp thru the tubes of standards and sample, accurate color comparisons can be made in the absence of daylight. Since the standards are prepared for use only at 13 mm depth of color, tubes of different diameter can not be used for accurate work.

DETERMINATION

Transfer 10 cc of the buffer substrate soln into a test tube 20 mm × 160 mm and add 0 5 cc of the milk to be tested. Add a few drops of $CHCl_3$, mix thoroly by rotating the tube, and cover to protect contents from dust. (Do not use rubber or cork stoppers; paper toweling placed over the open end of the tube is satisfactory.) Warm to 37–39° in a water bath and incubate at 34–37° for not less than 18 and not more than 24 hours. After incubation add 4.5 cc of the diluted Folin-Ciocalteu reagent. Mix, and allow to stand for 3 min. Filter, and transfer 5 cc of the filtrate to a test tube of 13 mm diam. Add 1 cc of the Na_2CO_3 soln and mix thoroly by rotating the tube. Place the tube in a boiling water bath for 5 min. and filter. Cool, and estimate the color of the filtrate by comparison with the permanent color standards.

CONTROL TEST

(To check deterioration of reagents and the presence of interfering substances in the milk sample)

To 10 cc of the buffer substrate soln, add 4.5 cc of the Folin-Ciocalteu reagent, and 0.5 cc of the milk sample. (Do not incubate.) Mix thoroly, allow to stand for 3 min., and filter. To 5 cc of the filtrate add 1 cc of the Na_2CO_3 soln, mix thoroly by rotating the tube, heat in a boiling water bath for 5 min., and filter. Cool, and compare the color of the filtrate with the permanent color standards. If the phenol value

obtained is greater than 0.02 mg, subtract the excess from the phenol value of the incubated sample to obtain the phenol value indicative of pasteurization treatment.

A phenol value of 0.04 mg of phenol per 0.5 cc of sample generally indicates milk heated to 143° F. for 30 min. A value greater than this indicates progressively inadequate heat treatment. In reporting results, give the mg. of phenol per 0.5 cc of sample as well as an interpretation as to whether the milk is pasteurized or underpasteurized.

(4) The tentative method for the determination of specific gravity of milk (p. 264, **3**) was changed to read as follows: "Determine specific gravity at 15.6/15.6° with a pycnometer or a standard hydrometer."

(5) The following method for approximating the total solids in milk was adopted as tentative:

TOTAL SOLIDS IN MILK
(Approximate)

Use the formula: $0.25\,L+1.2F$, in which $L=$ the lactometer reading at 15.6° (as determined by **3**, p. 264, times 1000 minus 1000); and $F=$ the percentage of fat in the milk.

(6) As revised last year, *This Journal*, **20**, 70 (1937), the official optical method for the determination of lactose in milk (pp. 266–7) was adopted as official (final action).

(7) The official method for preparation of sample for butter (p. 288) was clarified by the associate referee and now reads as follows:

PREPARATION OF SAMPLE

Soften the entire sample in a closed vessel by heating in a water bath kept at 39° with occasional shaking, until the temp. of the entire mass is 35°, or, by heating in a constant temp. oven kept at 35° until the temp. of the entire mass has reached the temp. of the oven. Shake vigorously until a perfectly homogeneous semi-solid mass is obtained. Weight the portion for analysis at once. If the sample is kept for any length of time, soften and prepare the sample again as directed above before withdrawing portions for analysis.

(8) The official indirect method for the determination of fat in butter (p. 289) was clarified by the associate referee to read as follows:

FAT

Take up the dry butter, obtained in the moisture determination in which no absorbent was used, 78, by mascerating with 15 cc of absolute ether or petroleum ether; transfer to a weighted Gooch crucible with the aid of a wash bottle filled with the solvent; and wash free from fat with 100 cc of the solvent. (The last 25 cc of the 100 cc of the solvent should pass thru the Gooch crucible without the aid of suction.) Dry the crucible and its contents at the temp. of boiling H_2O until the weight is constant. Repeat the washing with 25 cc of the solvent and dry to constant weight. Repeat this operation until there is no loss in weight due to the washing.

(9) The tentative method for the assay of vitamin D milk, *This Journal*, **20**, 78 (1937), was revised (see p. 90).

XXIII. EGGS AND EGG PRODUCTS

The following rapid method for the determination of the acidity of ether extract was adopted as tentative:

ACIDITY OF ETHER EXTRACT

Rapid Method

Prepare an aqueous alcoholic salt soln by dissolving 10 g of salt in about 50 cc of H_2O, adding 30 cc of 95% alcohol and diluting to 100 cc with H_2O.

Weigh out about 10 g of mixed whole liquid egg or 5 g of liquid yolks. Transfer to a suitable centrifuge bottle with 40 cc of the salt soln. Shake gently until the egg is thoroly mixed with the salt soln. Add 50 cc of ethyl ether followed by 50 cc of petroleum ether, stopper the bottle, and shake gently but thoroly until lipoids are extracted by the mixed ethers. (The color of the ether layer will be yellow when extraction of lipoids takes place.) If the ether layer is not yellow, shake more vigorously and rely on the centrifuge later to separate the layers. Centrifuge to separate the liquids. If a bad emulsion has formed, add 10 cc of ethyl alcohol, shake gently, and centrifuge again. Remove the mixed ether layer by carefully pouring off or blowing off with a wash bottle arrangement, or by any other method that will separate the ether layers. Repeat the extraction, using 30 cc each of ether and petroleum ether, and add to the first ether shakeout. If only acidity of the ether extract is to be determined, enough lipoids will be obtained by two extractions for titration purposes. If a complete extraction is desired, repeat the shaking with about 50 cc of mixed ethers, centrifuging, etc., until the ether layer is colorless after separation.

Evaporate the mixture of ethers from the first two extracts in a suitable dish on the steam bath. When the ether is removed, add 5 cc of absolute alcohol and evaporate again on the steam bath to aid in the removal of moisture. Dissolve the residual extract in a small amount of $CHCl_3$, filter into a tared beaker, and wash the dish and filter with $CHCl_3$. Evaporate off the $CHCl_3$ on the steam bath and continue heating a few minutes after the $CHCl_3$ is removed. Dry the beaker with a towel. Cool, and weigh.

Dissolve the residue in the beaker in neutral benzene and titrate with 0.05 *N* sodium ethylate with phenolphthalein as directed in the official method (p. 303, **25**).

Report acidity as cc of 0.05 *N* sodium ethylate per gram of ether extract.

XXIV. FISH AND OTHER MARINE PRODUCTS

The following methods were adopted as tentative:

FISH

1. PREPARATION OF SAMPLE

To prevent loss of H_2O during preparation and subsequent handling, do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in the following manner:

(a) *Fresh Fish.*—Clean and prepare in the usual manner. In the case of small fish (6 in. long or less), remove one longitudinal half from each of 5–10 fish. In the case of large fish, cut from at least 3 fish, 3 transverse slices, 1 in. thick; one slice from immediately back of the pectoral fins, one slice halfway between the first slice and the vent, and one slice immediately back of the vent.

Separate any bones that may be present as completely as possible from the pieces selected, leaving the skin intact so far as possible, since in many fish large quantities of fat are stored directly beneath the skin; pass rapidly thru a food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill the sample to inhibit decomposition.

(b) *Canned salmon and similar types of canned fish.*—Pass the entire contents of the tin thru a meat chopper 3 times, thoroly mixing each time.

(c) *Canned fish packed in oil.*—Drain the fish on a ¼ in. mesh sieve (or larger mesh that will retain all the meat) for 5 min. Return to the sieve any meat particles

passing thru. Prepare the solid portion as directed in (b). (The oil and brine may be separated and analyzed if desired.)

(d) *Shellfish other than oysters and scallops.*—If the sample is received in the shell, separate edible portions in the customary manner. Prepare the edible portion for analysis as directed in (b).

(e) *Fish packed wet in salt and brine.*—Drain off the brine and rinse off adhering salt crystals in a saturated salt soln. Drain again for 2 min. and proceed as directed in (a); in the case of sardine or anchovy types of small fish, as in (b).

(f) *Dried smoked or dried salt fish.*—Cut large samples into small pieces, mix, and quarter down to about ½ lb. Cut, shred, grind, or otherwise comminute the ½ lb. sample as finely as possible so that reasonable representative samples may be weighed for analysis after being thoroly mixed. (Duplicate or triplicate determinations may be necessary to establish the uniformity of the sample.)

2. ASH

Dry a sample representing about 2 g of dry material and proceed as directed under **8**, 336, at a temp. not to exceed 550°. If the material contains a large quantity of fat, make the preliminary ashing at a sufficiently low temp. to allow smoking off the fat without burning.

3. SALT

Weigh a suitably sized sample (depending on salt content) and proceed as directed in the methods for the determination of salt in oysters and scallops, *This Journal*, **20**, 71 (1937).

4. TOTAL NITROGEN

Proceed as directed under **II, 19, 22**, or **24**.

XXV. FLAVORING EXTRACTS

No additions, deletions, or other changes.

XXVI. FRUITS AND FRUIT PRODUCTS

No additions, deletions, or other changes.

XXVII. GRAIN AND STOCK FEEDS

(1) The Elmslie-Caldwell method for the determination of iodine in mineral feeds, *This Journal*, **18**, 338 (1935), was adopted as tentative.

(2) The qualitative tests for proteins (pp. 337–8, **10–17**) were adopted as official (final action).

(3) The qualitative (picrate) test for the determination of glucosides in feeds and similar materials (p. 347) was adopted as official (final action).

XXVIII. MEAT AND MEAT PRODUCTS

In the tentative method for the determination of starch in chopped meat, sausage, etc. (p. 357, **18**) "10% NaOH soln," in the 4th line from the end of the paragraph was changed to read "NaOH (1+1)."

XXIX. METALS IN FOODS

The colorimetric dithizone and the electrolytic methods for the rapid determination of lead on apples and pears (p. 391, **30–33**) were adopted as official (first action).

XXX. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

XXXI. OILS, FATS, AND WAXES

(1) The Fitelson method for the detection and estimation of tea seed oil, *This Journal*, **19**, 496 (1936), was made official (final action).

(2) The following modified Kaufmann method for the determination of the thiocyanogen number of fats and oils was adopted as tentative.

THIOCYANOGEN NUMBER OF FATS AND OILS

REAGENTS

(a) *Lead thiocyanate.*—Dissolve 331 g of the finest C.P. $Pb(NO_3)_2$ in 700 cc of H_2O and filter. Dissolve 194 g of C.P. KCNS in 500 cc of H_2O and filter. Add slowly the $Pb(NO_3)_2$ soln to the potassium thiocyanate solution with stirring, continue stirring for 30 min., and allow the precipitate to settle. Decant the supernatant liquid thru a filter paper on a Büchner funnel, using a slight suction, and wash the precipitate several times with H_2O by decantation. Then transfer the precipitate into the Büchner funnel, using a horn spoon and H_2O, and wash the precipitate with H_2O until the washings give no test for nitrates. Place the precipitate on a watch-glass and dry to constant weight (about 7 days) in a vacuum desiccator over H_2SO_4. The dried $Pb(SCN)_2$ should be white in color and should be stored in an air-tight brown bottle and kept in the dark. Yield about 260 g.

(b) *Thiocyanogen soln.*—Prepare anhydrous acetic acid by boiling gently for about 1 hour 500 cc of acetic acid, containing at least 99.5% of acetic acid, with 40 cc of acetic anhydride in a liter flask with a ground-in glass air condenser. Attach a $CaCl_2$ tube to the end of the condenser and allow the acetic acid to cool to room temp.

Solution 1:—Weigh 4.2 g of dry bromine into a 250 cc graduated flask; dissolve in 100 cc of pure dry CCl_4, and fill the flask up to the mark with anhydrous acetic acid.

Solution 2:—Pour 250 cc of anhydrous acetic acid on 12.5 g of the pure dry $Pb(S\,CN)_2$ in a white, dry, glass-stoppered liter bottle.

Add Soln 1 to Soln 2 in small quantities, giving Soln 2 a vigorous shaking after each addition and taking care that decoloration takes place before each new addition of soln.

After a complete mixture of Solns 1 and 2 has been obtained, allow the suspension, consisting of precipitated $PbBr_2$ and surplus $Pb(SCN)_2$ to settle. Filter the soln thru a dry filter paper into a dry, brown, glass-stoppered bottle. Keep the filtrate, which should be clear and colorless, or only slightly yellow, in the dark. This soln, if correctly prepared, will require 24–26 cc of 0.1 *N* thiosulfate soln for its iodometric titration. The thiocyanogen soln will keep about 1 week. After that time it begins to show a yellow color and a turbidity, and soon a fine yellow precipitate settles to the bottom of the bottle.

Determination

Weigh 0.1–0.2 g of the fat or oil (the excess of the thiocyanogen reagent should be 100–150%) into a 200 cc glass-stoppered bottle or flask. Add 25 cc of the thiocyanogen soln from a pipet, rotate the bottle gently until the fat is dissolved, and allow to stand in the dark 20–24 hours. Add 10 cc of 10% KI soln quickly and at one time, while shaking the bottle to avoid hydrolysis of the thiocyanogen soln. Add 100 cc of H_2O and titrate the liberated I with standardized 0.1 *N* thiosulfate soln in the usual manner, using starch indicator. Conduct at least two blank deter-

minations along with that on the sample. Substract the number of cc of the thiosulfate soln required by the sample from the number required by the blank. Multiply this number by the I equivalent of the thiosulfate soln. The value obtained is the quantity of I equivalent to the thiocyanogen absorbed by the fat or oil. Calculate the percentage by weight and report as the thiocyanogen number.

Using the thiocyanogen number together with the Hanus I number, calculate the composition of oils or fats composed of glycerides of oleic, linoleic, and total saturated acids by the following formulas:

$x+y+z=100-\%$ unsaponifiable matter;
$173.3\,x+86.1\,y=100$ (I No.);
$86.7\,x+86.1\,y=100$ (S CN No.), where
$x=\%$ linoleic acid glycerides;
$y=\%$ oleic acid glycerides; and
$z=\%$ saturated acid glycerides.

If the percentage of unsaturated acids present in an oil is known, calculate the percentages of oleic, linoleic, and linolenic acids present in the oil by the following formulas:

$273.7\,x+181.2\,y+89.9\,z=100$ (I No.);
$182.5\,x+90.6\,y+89.9\,z=100$ (S CN No.);
$x+y+z=\%$ unsaturated acids, where
$x=\%$ linolenic acid in oil;
$y=\%$ linoleic acid in oil; and
$z=\%$ oleic acid in oil.

(3) The official method for the determination of free fatty acids (p. 417) was deleted (first action).

(4) The N. C. P. A. methods for the determination of free fatty acids in crude and in refined oils were adopted as official (first action). The methods follow:

FREE FATTY ACIDS

N. C. P. A. Methods

(a) *In crude oils.*—Weigh 7.05 g of well-mixed oil into a 250 cc flask or 4 oz bottle. Add 2 cc of 1% alcoholic phenolphthalein soln to 50 cc of denatured alcohol Formula 30 (1 vol. of methanol and 10 vols. of 95% ethyl alcohol), and a sufficient quantity of NaOH soln to give a faint pink color. Add this mixture to the oil in the flask or bottle. Titrate with 0.25 *N* NaOH soln until a permanent faint pink color appears and persists for at least 1 min. with vigorous shaking of the soln. Report as percentage of free fatty acid expressed as oleic acid. The number of cc of the 0.25 *N* NaOH used in the titration corresponds to the percentage.

(b) *In refined oils.*—Put about 50 cc of alcohol (Formula 30) into a clean, dry 150 cc flask and add a few drops of refined oil and 2 cc of 1% phenolphthalein soln. Place the flask in H_2O at 60–65° until warm, and add a sufficient quantity of the NaOH soln to produce a faint permanent pink color. Weight 56.4 of the refined oil into the neutralized alcohol and titrate, occasionally warming and violently shaking the mixture until the same faint permanent pink color appears in the supernatant alcohol. Multiply the number of cc of 0.1 *N* NaOH by 0.05 and report as percentage of free fatty acid expressed as oleic acid.

XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

No additions, deletions, or other changes.

XXXIII. SPICES AND OTHER CONDIMENTS

(1) The official method for the determination of ash in vinegar (p. 456, **58**) was deleted (first action).

(2) The following method for the determination of ash in vinegar was adopted as official (first action).

ASH IN VINEGAR

Measure 25 cc of the vinegar into a weighed Pt dish, evaporate to dryness on a water or steam bath, and heat in a muffle between 500 and 550° for 30 min. Break up the charred mass in the platinum dish, add hot H_2O, filter thru an ashless filter, and wash *thoroly* with hot H_2O. Return the filter and contents to the dish, dry, and heat between 500 and 550° for 30 min., or until all the carbon is burned off. Add the filtrate, evaporate to dryness, and heat between 500 and 550° for 15 min. Cool in a desiccator and weigh (Weight A). Reheat in the muffle between 500 and 550° for 5 min., and cool for not more than 1 hour in a desiccator containing an efficient desiccant. Put no more than 2 dishes, preferably only 1, in a desiccator at one time. Place Weight A on the balance pan before removing the dish from the desiccator, and weight rapidly to a milligram. Calculate total ash from the last weight.

(3) In line 3 of **67**, p. 457, **57** was changed to **66**.

XXXIV. SUGARS AND SUGAR PRODUCTS

(1) The official method (Wein) for the determination of maltose (p. 484, **54**, **55**) was deleted (first action).

(2) The following vacuum drying method of the International Commission for Uniform Methods of Sugar Analysis was made official (first action):

MOISTURE

Vacuum Drying Method

(Applicable to cane and beet raws and refined sugar.)

Dry 2–5 g of the prepared sample **1(a)** in a flat dish of Ni, Pt, or Al and with a tight-fitting cover, at a temp. not exceeding 70° (preferably at 60°), under a pressure not exceeding 50 mm of Hg, for 2 hours. Remove the dish from the oven, put cover in place, cool in a desiccator, and weigh. Re-dry for an hour and repeat the process until the change in weight between successive weighings at 1 hour intervals is not more than 2 mg. (The oven should be bled with a current of dry air during drying to insure removal of the water vapors.)

(3) The International Scale of Refractive Indices of Sucrose Solutions at 20° C, 1936, *Intern Sugar J. Supplement*, **39**, 1–40 (1937), was adopted as official (first action).

(4) The International Temperature Correction Table, 1936, *Ibid.*, was adopted as official (first action).

XXXV. VEGETABLES AND VEGETABLE PRODUCTS

(1) The following method for the determination of alcohol-insoluble material in canned peas was adopted as official (first action):

ALCOHOL-INSOLUBLE MATERIAL IN CANNED PEAS

Pour the sample on an 8-mesh screen, using an 8-in. screen for containers of less than 3 lbs. net weight, and a 12-in. screen for larger containers. Spread the peas evenly and allow to drain. Transfer the peas to a white pan and remove any foreign material. Add a volume of water equal to double the volume of the original sample.

Pour the peas back on the screen, spreading evenly; tilt the screen as much as possible without shifting the peas; and drain for 2 min. With a cloth wipe surplus moisture from the lower surface of the screen. Grind the drained peas in a food chopper, stir until homogeneous, and weight 20 g of the ground material into a 600 cc beaker. Add 300 cc of 80% alcohol (by volume), stir, cover beaker, and bring to a boil. Simmer slowly for 30 min.

Fit into a Büchner funnel, a filter paper previously prepared (place a paper of appropriate size in a flat-bottomed dish, uncovered but provided with a tight-fitting cover. Dry for 2 hours at the temp. of boiling H_2O, cover dish, cool in a desiccator, and weigh at once).

Transfer the contents of the beaker to a Büchner funnel, filter with suction, and wash the material on a filter with the 80% alcohol until the washings are clear and colorless.

Transfer the filter paper and alcohol-insoluble solids to the dish used in the preparation of the filter paper, dry uncovered for 2 hours at the temp. of boiling H_2O, place cover on the dish, cool in a desiccator, and weigh at once. From this weight deduct the weight of the dish, cover, and paper. Calculate this weight to percentage.

(2) The method adopted as tentative last year, *This Journal*, **20**, 78 (1937), for the determination of chlorides in tomato juice, was adopted as official (first action).

XXXVI. VITAMINS

The tentative method for the assay of vitamin D milk, *This Journal*, **20**, 98 (1937), was revised to read as follows.

VITAMIN D MILK

COLLECTION AND PRESERVATION OF THE SAMPLE

Unless the sample of milk, in the original bottle, can be delivered to the assayer immediately after collection, store it under refrigeration until delivered. Make shipment to the assayer in an iced container. After acceptance by the assayer, preserve the milk in its original homogeneous state, for a period of not more than 1 month, by the addition of 2 drops of 10% formalin and suitable refrigeration.

PROCEDURE

As the basic procedure follow the method of assay for vitamin D in cod liver oil described in the Pharmacopoeia of the United States XI, 1937 Supplement, except that the statement given below for the vitamin D potency of the milk shall replace that given on p. 97 of the Supplement, and headed "*Vitamin D potency of the assay oil.*"

Feed the calculated quantities of U. S. P. Reference Oil and of the sample of milk and vary the assay period according to the following options, but feed the U. S. P. Reference Oil and the milk sample according to the same plan:

1. Proceed according to the U. S. P. XI, 1937 Supplement method.
2. Feed the supplements on the first day or in equal portions on each of the first 3 or 8 days of a 10-day assay period.
3. Feed the supplements on the first day or in equal portions on each of the first 3 or 5 days of a 7-day assay period.
4. Feed the supplements admixed with the quantity of basal ration that will be comsumed in 7 or 8 days. Feed the unsupplemented basal ration during the remainder of a 10-day assay period.
5. Feed the supplements admixed with the quantity of basal ration that will be

consumed in 4 or 5 days. Feed the unsupplemented basal ration during the remainder of a 7-day assay period.

Vitamin D Potency of Milk.—In determining the vitamin D potency of the milk, the average performance of the reference oil group with respect to the healing of the rachitic metaphysis shall be such that two-thirds or more, but not less than 7, of the animals of this group show macroscopic evidence of calcification. When the average response of the assay group is equal to or greater than that of the reference oil group, the vitamin D content of the milk fed during the assay period is equal to, or greater than the vitamin D content of the reference oil fed during the assay period. When the average response of the assay group is less than that of the reference group, the vitamin D content of the milk fed during the assay period is less than the vitamin D content of the reference oil fed during the assay period. The data from a rat shall be considered valid for establishing the average performance of a group only on the condition that the weight of the rat at the termination of the assay period shall equal or exceed the weight of the rat on the beginning day of the assay period, and on the condition that the rat has consumed each prescribed dose of milk within 24 hours from the time it was fed.

Evaporated and Dried Milks.—Evaporated or dried milk may be incorporated with the basal ration (paragraphs 4 and 5) or diluted to original volume (paragraphs 1–3).

EXPLANATORY NOTES.—Experimental trials and the experience of those now conducting routine assays of vitamin D milk show that departure from the U. S. P. XI method may be made in the manner in which the milk sample and the reference oil are fed and in the length of the assay period.

If it is necessary to feed a relatively large quantity of milk, a single feeding, as suggested below, may not be possible, and daily feedings for a period of days are necessary.

The extent of calcification at the end of a 7-day assay period is not so marked as at the end of the 10-day period. Hence, it may be necessary to feed higher levels of milk and reference oil to obtain an adequate response at the end of a 7-day period.

In paragraphs 2 and 3 several options are given as to the number of days during which supplements may be fed. These periods have been indicated because of the experimental data on their use already accumulated and for the sake of the greater uniformity that will prevail than if the number of days of feeding were entirely optional.

XXXVII. WATERS, BRINE, AND SALT

The statement in **14(c)**, p. 506, "0.0001 mg of N as NO_2," was changed to read, "0.0001 mg of N" (first action).

XXXVIII. RADIOACTIVITY

No additions, deletions, or other changes.

XXXIX. DRUGS

(1) The following microchemical methods for the identification of apomorphine, benzylmorphine (peronine), ethylmorphine (dionine) and hydrastinine were adopted as tentative:

APOMORPHINE, BENZYLMORPHINE (PERONINE), ETHYLMORPHINE (DIONINE), AND HYDRASTININE

REAGENTS

Gold chloride soln.—Prepare as directed in **176(j)**, p. 602.

Hydrochloric acid soln.—5%.

Potassium permanganate soln.—Dissolve 1 g of $KMnO_4$ in 100 cc of H_2O.

Mercuric chloride soln.—Prepare as directed in 176(e), p. 602.

Wagner's reagent.—Prepare as directed in 176(c), p. 602.

Potassium iodide soln.—Prepare as directed in 176(d), p. 602.

Ammonium thiocyanate soln.—Dissolve 5 g of NH_4CNS in 100 cc of H_2O.

Potassium ferrocyanide soln.—Dissolve 5 g of $K_4Fe(CN)_6 \cdot 3H_2O$ in 100 cc of H_2O and use only when freshly prepared.

IDENTIFICATION

Place a drop of the alkaloidal soln on a clean, glass slide, and add a drop of reagent by means of a clean, glass rod. Without covering, examine under the micro-

Characteristics of microchemical tests for alkaloids

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Apomorphine	Gold chloride	Red-brown; fine needles; in dense masses in all solns to 1:10,000
	Hydrochloric acid	1:50. Small rods, singly and in clusters
Hydrastinine	Potassium permanganate	1:500. Immediate red plates often with serrated edges. In concentrated soln, great number of large red or brown plates with deeply cut edges
	Mercuric chloride	1:500. Transparent needles forming branches rapidly in neutral and acidified solns
	To one drop of 5% hydrochloric acid add the potassium ferrocyanide soln	1:200. Yellow rhombic plates and tree-like crystals
Ethylmorphine (Dionine)	Wagner's reagent	1:200. Groups of yellow needles, branching later
	Mercuric chloride	Transparent plates often with notched ends. Singly and in groups. Stir to start crystallization
Benzylmorphine (Peronine)	Potassium iodide	1:200. Dense rosettes of needles. Crystals are formed readily in dilute solns (1:1000) in form of sheaves of needles
	Ammonium thiocyanate	1:200. Rosettes and sheaves of needles in acid or neutral soln
	Hydrochloric acid	1:100. Rods, usually notched at ends and often in rosettes, are formed on stirring

scope, using low power (magnification of 100–150 is suitable). Note the kind of crystals formed and compare their characteristics with a control specimen of the alkaloid of the same dilution.

(2) The following microchemical methods for the identification of acetylsalicylic acid, benzoic acid, and salicylic acid were adopted as tentative:

ACETYLSALICYLIC ACID, BENZOIC ACID, AND SALICYLIC ACID

REAGENTS

(a) *Zinc pyridine soln.*—Add 1 cc of pyridine to a soln of 1 g of Zn acetate in 20 cc of H_2O.

(b) *Lead triethanolamine soln.*—Add 1 cc of triethanolamine (Technical 90% is satisfactory) to a soln of 1 g of neutral Pb acetate in 20 cc of H_2O. A slight turbidity does not interfere.

(c) *Silver nitrate soln.*—Dissolve 1 g of $AgNO_3$ in 20 cc of H_2O.

Characteristics of microchemical tests for synthetics

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Acetyl-salicyclic acid	2% triethanolamine	1:50	Silver nitrate	Fine curling hair-like crystals form first near the edge of the drop
Salicylic acid	dry powder		Bromide-bromate soln	Stir a few crystals of the synthetic into a drop of the HCl Add a drop of the bromide-bromate soln. Fine needles appear to grow from the crystal of salicylic acid
	dry powder		Lead triethanolamine	Stir a few crystals into a drop of the reagent. Rods or needles grow from the crystal of salicylic acid
	2% triethanolamine	1:100 to 1:200	Silver nitrate	Small irregular plates; a few short rods
Benzoic acid	dry powder		Lead triethanolamine	Stir a small quantity of the synthetic into a drop of reagent. Stir thoroly to induce crystallization. 4-sided plates, singly and in groups.
	dry powder		Zinc pyridine	Stir a small quantity of the synthetic into a drop of reagent. Stir thoroly to induce crystallization. Hexagonal crystals
	2% triethanolamine	1:100	Silver nitrate	Rods or curving blades with irregular ends

(d) *Triethanolamine soln.*—2%. Dissolve 2 cc of triethanolamine in H_2O to make 100 cc.

(e) *Hydrochloric acid soln.*—10%.

(f) *Bromide-bromate soln.*—Prepare as directed in **26**(c), p. 551.

PREPARATION OF SAMPLE

Separate the compound for microchemical testing in pure form. Prepare a soln of a portion of the purified compound with the solvent specified for the individual synthetic.

Control.—For comparison treat a known sample as directed in the tests.

IDENTIFICATION

To a drop of the soln of the compound or to about 0.001 g of the powder on a glass slide, add a drop of the specified reagent. Do not stir unless directed. Without covering, examine for crystal formation under the microscope, using about 100 magnification. Observe the characteristics of the crystals found and compare with controls and description.

(3) The following method for the determination of free iodine in iodine ointment was adopted as tentative:

FREE IODINE IN IODINE OINTMENT

REAGENT

Potassium arsenite soln.—Dissolve 4.948 g of arsenic trixoide in a concentrated soln of NaOH (4 g+4 cc of H_2O), add 100 cc of a saturated aqueous soln of $KHCO_3$, and make to 1000 cc with H_2O.

DETERMINATION

Weigh accurately 4–5 g of the ointment in an iodine flask, stopper, and heat on a water bath until the sample is just fluid. Add 30 cc of $CHCl_3$ and shake with a rotary motion until the base is dissolved. Add 30 cc of H_2O and titrate immediately with 0.1 *N* $KAsO_2$ soln (containing sufficient $KHCO_3$ to neutralize the HI formed), using starch as the indicator. 1 cc of 0.1 *N* $KAsO_2$ = 0.012692 g of I.

(4) The following method for the determination of pyridium was adopted as tentative:

PYRIDIUM

REAGENTS

(a) *Standard titanium trichloride soln.*—Prepare as directed in **37**, p. 254, and standardize as directed in **38**, Method II, p. 255.

(b) *Light green S F yellowish soln.*—Dissolve 1 g in H_2O and dilute to 1000 cc.

PREPARATION OF SAMPLE

Solutions.—To a volume containing about 0.1 g of pyridium, add 10 cc of 0.1 *N* HCl and dilute to 100 cc.

Tablets and jelly.—Weigh a quantity of the sample (powdered in the case of tablets) equivalent to about 0.1 g of pyridium, add 10 cc of 0.1 *N* HCl, and dilute to 100 cc.

Ointments.—Weigh in a 100 cc beaker a portion of the sample equivalent to about 0.1 g of pyridium, stir with ether until the ointment base is dissolved, and wash into a separator with ether and H_2O. Shake thoroly and draw off the aqueous layer into a third separator containing 25 cc of ether. Shake, and draw off the aqueous layer into a second separator containing 25 cc of ether. Shake, and transfer the

aqueous layer to a 250 cc beaker. Wash the ether layers with alternate 10 cc portions of HCl (1+1) and H_2O until no more color is removed, passing each portion of HCl or H_2O successively thru the three separators and finally into the beaker. Nearly neutralize the combined acid extracts with NH_4OH, cool, wash into a separator, make ammoniacal, and extract with 25 cc portions of $CHCl_3$ until no more color is removed, filtering the $CHCl_3$ thru a pledget of cotton in the stem of the separator. Evaporate the combined $CHCl_3$ extracts just to dryness, take up in 10 cc of 0.1 *N* HCl, and dilute to 100 cc.

DETERMINATION

Heat the soln to boiling, add 15 g of Na acid tartrate, and boil 2 min. Add 10 cc of light green S F yellowish soln and titrate hot with the standard $TiCl_3$ soln in a current of CO_2. The end point is the change from green to pale yellow. Run a blank titration with 10 cc of 0.1 *N* HCl, 90 cc of H_2O, 15 g of Na acid tartrate, and 10 cc of the light green S F yellowish soln, and subtract from the volume of $TiCl_3$ previously found. 1 cc of 0.1 *N* $TiCl_3$ = 0.006240 g of pyridium ($C_{11}H_{11}N_5 \cdot HCl$).

(5) The following method for the determination of cinchophen in the presence of salicylates was adopted as tentative:

CINCHOPHEN IN PRESENCE OF SALICYLATES

REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 12.5 g of monohydrated Na_2CO_3 in sufficient H_2O to make 100 cc.

(b) *Iodine soln.*—0.1 *N*. Prepare and standardize as directed in 3(c), p. 542.

(c) *Sodium thiosulfate soln.*—0.02 *N*. Prepare and standardize as directed in 3(b), p. 542.

(d) *Starch indicator.*—Prepare as directed in 3(e), p. 41.

DETERMINATION

If the product is a solid, weigh into a 50 cc beaker sufficient finely powdered sample to contain about 0.15 g of cinchophen. Treat with 5, 3, and 3 cc portions of the Na_2CO_3 soln, and filter thru a small (5 cm) paper into a 50 cc beaker, finally washing the first beaker and the paper with a little H_2O. Evaporate the filtrate and washings to complete dryness on a steam bath with the aid of an air blast. If the product is in the form of a clear soln, transfer a measured portion to a beaker, and evaporate to dryness. In either case, dissolve the hot residue in 5 cc of glacial acetic acid and transfer to a 100 cc volumetric flask, using not more than 10 cc of the acid to complete the transfer. Heat to about 90° on the steam bath. Add 25 cc of the I soln slowly from a pipet with constant agitation of the flask, and immediately stopper the flask. Allow to cool, dilute to 100 cc with H_2O, stopper, and let stand with occasional thoro agitation for 30 min. Filter thru a small, rapid filter, rejecting the first 15 cc of filtrate and immediately titrate a 50 cc aliquot with the standard thiosulfate soln, adding the starch indicator as the end point is approached.

1 cc of 0.1 *N* I = 0.01661 g of cinchophen ($C_{16}H_{11}O_2N$).

(6) The following method for the determination of homatropine in tablets was adopted as tentative:

HOMATROPINE IN TABLETS

REAGENTS

(a) *Wagner's reagent.*—Prepare as directed in 176(c), p. 602.

(b) *Methyl red indicator.*—Dissolve 0.2 g of methyl red in 100 cc of neutralized alcohol.

PREPARATION OF SAMPLE

To determine the average weight per tablet, count and weigh 100 whole tablets or a number representative of the lot. Powder at least 20 tablets in a mortar and pass thru a 60-mesh sieve. Mix thoroly.

DETERMINATION

Weigh accurately a quantity of sample equal to about 0.130 g of the alkaloidal salt, and transfer to a separator. Dissolve in 10–20 cc of H_2O and add 2 cc of NH_4OH. Add about 20 cc of $CHCl_3$, agitate, and allow to stand until separation is complete. Draw off the $CHCl_3$ layer into a second separator and repeat the extraction with fresh portions of the solvent until the alkaloid is completely removed (5 extractions usually suffice). Test for complete removal of the alkaloid with Wagner's reagent. After combining all the fractions, wash the combined $CHCl_3$ solns by agitation with 5 cc of H_2O and allow to settle until separation is complete. Filter the $CHCl_3$ soln thru a pledget of absorbent cotton in a funnel into a small beaker. Add 10 cc of $CHCl_3$ and agitate; when all the H_2O has risen to the surface, draw off the $CHCl_3$ and filter into the beaker. Wash the outer surface of the stem of the separator and the funnel and its stem with a little $CHCl_3$, adding the washings to the beaker. Evaporate the soln on the steam bath to about 5 cc. Add a measured excess volume of 0.02 *N* H_2SO_4. Place the beaker in a warm place and evaporate with the aid of a fan until the odor of $CHCl_3$ has disappeared. Cool the solution and titrate back with 0.02 *N* NaOH, using 1 drop of methyl red indicator.

1 cc of 0.02 *N* H_2SO_4 = 0.007122 g of homatropine hydrobromide
or 0.006233 g of homatropine hydrochloride.

(7) The following methods for the determination of caffeine and of potassium bromide in effervescent potassium bromide with caffeine were adopted as tentative:

EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE

REAGENTS

(a) *Silver nitrate soln.*—0.1 *N*. Prepare and standardize as directed in **52(a)**, p. 33.

(b) *Ammonium or potassium thiocyanate soln.*—0.1 *N*. Adjust by titrating against the 0.1 *N* $AgNO_3$.

(c) *Ferric ammonium sulfate indicator.*—Dissolve 8 g of $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ in sufficient H_2O to make 100 cc.

(d) *Nitric acid.*—Concentrated, C.P.

PREPARATION OF SAMPLE

Powder the sample, transfer immediately to a dry bottle, and seal tightly. Thoroly mix the powder in the bottle by rotating and shaking before removal of sample for analysis. Weigh out all needed portions as nearly at the same time as possible. Avoid extreme temperatures and humidities when opening and storing samples.

DETERMINATIONS

Potassium bromide.—Weigh 2.5–3 g of the preparation and transfer to a 500 cc Erlenmeyer flask. Add 200 cc of H_2O and swirl gently, avoiding loss of soln by spattering. Acidify the soln with the HNO_3 and then add 5 cc in excess. Add 50 cc of the $AgNO_3$ soln and 2 cc of the indicator. Allow the mixture to stand several minutes and swirl occasionally as an aid in flocculating the AgBr. Titrate the excess of $AgNO_3$ with the thiocyanate soln. 1 cc of 0.1 *N* $AgNO_3$ = 0.01190 g of KBr.

Caffeine.—Weigh 12–15 g of the sample, transfer to a separator, and slowly add 50 cc of H_2O, avoiding loss of soln by spattering. If the soln is not alkaline to litmus, make basic with the NaOH soln. Add 50 cc of $CHCl_3$, shake vigorously, and after clearing, draw off the lower layer thru a small filter, previously moistened with $CHCl_3$, into a beaker. Repeat the extraction twice, using 50 cc portions of the $CHCl_3$ for each extraction. Wash the filter and funnel with a few cc of $CHCl_3$ to remove any adhering caffeine. Evaporate the combined $CHCl_3$ filtrate on a water bath to about 10 cc, finally transferring the residual liquid by washing with $CHCl_3$, to a small weighed beaker. Allow the soln to evaporate by gentle heat and an air blast. Dry the residue to constant weight at 80°, and weigh as anhydrous caffeine.

ENZYMES

The following method for the determination of the proteolytic activity of papain was adopted as tentative:

PROTEOLYTIC ACTIVITY OF PAPAIN

PREPARATION OF SAMPLE

Unactivated.—If the enzyme preparation is a solid, finely divide it by grinding to a smooth paste in a small mortar with a little freshly boiled cold H_2O. Then suspend the enzyme in cold boiled H_2O in the proportion of 10 mg of original preparation per cc. After 5–10 min. centrifuge the suspension and discard the sediment.

Activated.—Proceed as directed for the unactivated preparation but use half-saturated H_2S water instead of boiled water. After centrifuging, incubate the enzyme soln at 40° for 1 hour to complete the activation.

REAGENTS

(a) *Casein soln.*—Make a 6% soln of Hammarsten's casein by rubbing up 60 g of this casein with a little H_2O in a mortar and gradually adding 60 cc of 1 *N* NaOH and H_2O until the volume totals 1 liter. Heat the viscous soln for 30 min. in a bath of boiling H_2O, cool, and filter (glass wool) if necessary.

(b) *Buffer soln.*—Prepare 0.2 *M* monosodium citrate soln by partial neutralization of citric acid with NaOH.

(c) *Titrating soln.*—0.1 *N* alcoholic KOH.

(d) *Indicator.*—1% alcoholic soln of thymolphthalein.

PROCEDURE

Have prepared a constant temperature water bath at 40°.

(a) Place 10 cc of the casein soln and a small charge of 4 mm diam. glass beads in each of several 125 cc glass-stoppered bottles and bring the bottles and contents to 40°. Add the desired volume of the prepared enzyme soln but do not use more than 4 cc. If this quantity is insufficient (see later), prepare a more concentrated soln of the enzyme. Add immediately exactly 3 cc of the buffer soln (the *p*H of the system should then be 5.0 ±0.1). Shake the bottle vigorously for a few seconds and replace in the thermostat.

Incubate the mixture for 20 min. at 40°, counting the time from the addition of the buffer. Add 1 cc of the indicator and begin titrating with 0.1 *N* alcoholic KOH. As soon as a deep blue color appears shake the bottle until the color is discharged or the precipitate is completely dissolved. (It is usually best to add the alkali in doses of about 0.5 cc at a time.) When all the precipitated casein has been brought into soln, transfer the contents of the bottle to a 400–500 cc flask and rinse out the bottle 2 or 3 times with 95% alcohol, using a total of 25 cc for this purpose. Add sufficient alcoholic KOH to restore the blue color in the titration, then add 175 cc

of hot (boiling) alcohol. Carefully add more alcoholic KOH until a pale but distinct blue color persists in the soln.

Make a control titration exactly as described, but do it immediately after the addition of the buffer and therefore without any incubation time. The difference in time between the titration of the undigested sample and that of the digested sample is a measure of the proteolytic activation of the enzyme.

CALCULATION OF THE PROTEINASE UNIT

For smaller quantities of enzyme the extent of hydrolysis determined by the titration described is a straight line function of the amounts of papain used. For accurate work determine this straight line by making several titrations with different quantities of enzyme. If the quantities of papain used are too large, the straight-line relationship will no longer hold; if they are too small the determination will be correspondingly inaccurate. Quantities of enzymes giving titration differences of 0.6–1.2 cc of 0.1 *N* KOH are recommended.

The unit of papain may be considered the quantity of enzyme that produces, under the conditions outlined, a titration difference of 1 cc of 0.1 *N* KOH, determined either graphically or arithmetically. The value of the original preparation is then expressed in units per mg, or as mg of the papain preparation necessary to make one unit.

APPENDIX I. STANDARD SOLUTIONS

No additions, deletions, or other changes.

APPENDIX II. DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

See p. 58 for the definitions adopted as official this year.

No report was given by the Committee on Standard Scale for Immersion Refractometer.

REPORT OF COMMITTEE TO COLLABORATE WITH THE AMERICAN PUBLIC HEALTH ASSOCIATION ON METHODS OF MILK ANALYSIS

Your Committee has prepared a revision of the chemical section of "Standard Methods of Milk Analysis" of the American Public Health Association for the 7th edition of that publication.

The revision conforms to the text of the 4th edition of *Methods of Analysis* of our Association as pertains to the analysis of milk, cream, and ice cream.

It will be possible for your Committee to hold the manuscript of the revision long enough to include any changes or additions adopted at this A.O.A.C. meeting relating to the subject matter of that text.

Two methods in which members of the American Public Health Association are particularly interested, and which they are anxious to have included in the forthcoming edition of their methods, are the phosphatase

test for pasteurized milk and a bioassay method for vitamin D milk. Both of these topics are of interest to our Association also, and they are subjects assigned to duly appointed associate referees for report at this meeting. Your Committee will be governed by the action taken on the recommendations made.

The Committee gratefully acknowledges the cooperation of our associate editor, Miss Lapp, and of Dr. Fisher of the Agricultural Experiment Station in New Haven, in preparing the revision.

E. M. Bailey, *Chairman*
G. G. Frary
F. C. Blanck

Approved.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE

The Crop Protection Institute continues to pursue the same policies and kinds of research as outlined in previous reports. During the past year the Institute has maintained sixteen projects. Most of these are full time projects, but some are of a preliminary or exploratory character.

The work has been distributed in seventeen states. Eighteen men have been employed. Eight of the projects have been largely in the field of entomology, nine in plant pathology and plant nutrition, and one on an animal disease problem.

There has been a total of $53,735.00 provided by commercial organizations for financing these research projects.

The Institute has issued the following bulletins giving reports on the projects:

No. 52—Sulphuric Acid for Control of Weeds, by W. E. Ball and O. C. French.
No. 54—The Role of Pine Oil in Cattle Fly Sprays, by A. M. Pearson.
No. 55—Copper Sulphate as a Plant Nutrient and Soil Amendment, by W. L. Churchman, R. Russell, and T. F. Manns.
No. 56—The Crop Protection Institute, its Organization, Plan of Procedure and Work Accomplished, by W. C. O'Kane.
No. 57—Ovicidal and Scalicidal Properties of Solutions of Dinitro-O-Cyclohexyl Phenol in Petroleum Oil, by J. F. Kagy and C. H. Richardson.
No. 58—Toxicity of Some Nitro-Phenols as Stomach Poisons for Several Species of Insects, by J. F. Kagy.
No. 59—Laboratory Methods of Comparing the Toxicity of Substances to San José Scale, by J. F. Kagy.
No. 60—Halowax (Chlorinated Naphthalene) as an Ovicide for Codling Moth and Oriental Fruit Moth, by E. P. Breakay and A. C. Miller.

H. J. Patterson
W. H. MacIntire

Approved.

REPORT OF SECRETARY-TREASURER

We have had a splendid attendance this year, the registration being about 500.

The enlarged program also attests to the growth of the Association. Last year, with a 16-page program, we could supply a number of pages for your personal notes, but this year it was necessary to fairly "squeeze the type" in order to have even a part of one page for this purpose.

More reports and papers were given this year than at any previous meeting. There is also a livelier interest in every phase of our work, as shown by the alertness of referees, associate referees, and committees, and also by the letters received at this office from all parts of the world.

The business affairs of the Association have progressed with little friction this year. After a revised edition of *Methods of Analysis* is completed and the accumulated orders are disposed of there is seemingly a breathing spell during which all the loose ends can be caught up. Such were the conditions during 1937. With our present system of recording orders and mailing there is little delay in filling the needs of all our members and customers.

A few appointments to refereeships were made after the general list had been published in the February *Journal*. They are the following: E. W. Campbell, of Augusta, Maine, was appointed in place of C. D. Howard, resigned, for the new work the Association is assuming on cosmetics; Louis Ehrenfeld, of Chicago, Illinois, was appointed as Associate Referee on Diastatic Power in Malt; and F. W. Gilcreas, Albany, New York, was appointed Associate Referee on Tests for Pasteurization of Dairy Products. The name of Anna E. Mix, as Associate Referee on Benzoate of Soda, was inadvertently omitted from the general list. One resignation was received, that of Dr. C. L. Hare, Auburn, Alabama, member of the Executive Committee, who finds that his health will not permit him to continue this work. A telegram expressing the good wishes of the Association and hopes for a speedy recovery was sent to Dr. Hare. A telegram was also sent to Dr. Frank E. Shutt, Agricultural Experiment Station, Ottawa, Canada, expressing the continued good wishes of the Association upon his retirement from active duty.

Two regular attendants and active workers at our meetings, G. L. Bidwell and O. B. Winter, passed on during the year, as did also P. F. Trowbridge, a past president of the Association and a most energetic member in the earlier days of the organization. The Committee on Necrology will report more fully on the serious loss to the Association of these worthy members. The vacancy on the Special Committee on Moisture occasioned by Mr. Bidwell's death was filled by the appointment of P. A. Clifford.

The Executive Committee at a meeting Sunday evening discussed what

might be done to increase interest in the work of the Association, since the organization is now financially in a position to take some action along these lines. The Committee decided to provide for a committee that would bring in a report at the next meeting on a plan for an A.O.A.C. award. Just what form such a project will take will be determined next year. The Committee is authorized to study a plan for an endowed fellowship or an allotment for special study of some method that does not lend itself to the usual referee type of collaborative study. (Later the President announced the following members to constitute this Committee: W. H. MacIntire, of Tennessee, W. B. White, of Washington, and E. M. Bailey, of Connecticut.)

The Executive Committee authorized the expenditure of a limited amount of funds in having translated and printed in our *Journal* certain articles of special merit. The Committee also authorized the Secretary-Treasurer to expend not to exceed $500 in the publication of a valuable bibliography on phosphoric acid prepared by Dr. R. N. Brackett some years ago as a part of a scheme of publishing bibliographies on publications of interest to this Association. This document has been used by some members, and it is proposed that 1,000 to 1,500 reprints be ordered, for which a small fee will be charged to cover cost of publication.

After presentation yesterday and discussion today the following changes were made in the By-laws of the Constitution:

Paragraph 5 was changed to read as follows:

> No method shall be adopted as official until it has received collaborative study and has been recommended by the appropriate referee at two annual meetings, and no official method shall be amended until such change has been recommended by the appropriate referee at two annual meetings.

The following additional by-law was adopted.

> No official method shall be repealed until such action has been recommended by the appropriate referee at two annual meetings.

The financial statement follows:

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR THE YEAR ENDED SEPTEMBER 30, 1937

Balance, October 1, 1936:		
Lincoln National Bank	$ 4,376.80	
Montgomery Building Association	52.50	$ 4,429.30

RECEIPTS

Sales:		
Methods	$12,120.70	
Journals	4,539.75	
Reprints	146.50	
Wiley's Principles	45.00	
	$16,851.95	

Less: Discounts Allowed	$1,913.98		
Refunds	24.55	1,938.53	
Net Sales			14,913.42
Other Income:			
Advertisements		$ 75.00	
Interest Income:			
U. S. Treasury Bonds	$ 190.00		
Federal Land Bank Bonds	97.50		
Home Owners Loan Corporation Bonds	27.50		
Montgomery Building Association	29.36	344.36	
Total Other Income			419.36
Miscellaneous Receipts:			
Books ordered through Association		$ 471.30	
Commercial National Bank, Liquidating Dividend		24.08	
Returned Checks Made Good		29.00	
Federal Land Bank Bonds Redeemed		1,000.00	
Total Miscellaneous Receipts			1,524.38
			$21,286.46

DISBURSEMENTS

Expenses:			
Salaries		$ 1,127.00	
Postage		660.00	
Meeting and Association Expenses		571.47	
Stationery and Supplies		119.50	
Auditing		80.00	
Premiums, Employees' Bonds		12.50	
Safe Deposit Rental		3.30	
Exchange		5.02	
Printing and Binding:			
Reprints	$ 431.35		
Journals	4,064.41		
Methods	6,309.95	10,805.71	
Insurance		10.10	
Freight and Express		182.28	
Total Expenses			$13,576.88
Miscellaneous Disbursements:			
Books Ordered through Association		$ 489.15	
Returned Checks		66.70	
Furniture and Equipment		97.26	
2 U. S. Treasury Bonds		2,024.60	
Total Miscellaneous Disbursements			2,677.71

Balance, September 30, 1937:		
Lincoln National Bank	$ 4,950.01	
Montgomery Building Association	81.86	5,031.87
		$21,286.46

BALANCE SHEET AS AT SEPTEMBER 30, 1937

ASSETS

Current Assets:			
Cash in Banks:			
Lincoln National Bank	$4,950.01		
Montgomery Building Association	81.86	$ 5,031.87	
Accounts Receivable	$3,307.01		
Less: Reserve for Doubtful Accounts	120.80	3,186.21	
Inventories		6,424.62	
Total Current Assets			$14,642.70
Investments:			
Home Owners Loan Corporation Bonds		$ 1,000.00	
U. S. Treasury Bonds		7,000 00	
Federal Land Bank Bonds.		3,000.00	
Total Investments			11,000.00
Cash in Closed Banks:			
Federal-American Bank and Trust Company		$ 36 93	
Commercial National Bank		96 34	133.27
Furniture and Fixtures			97.26
Total Assets			$25,873 23

SURPLUS

Balance, October 1, 1936	$21,866.44	
Add: Net Profit for the Year.	4,048.49	
	$25,914.93	
Less: Adjustment for Returned Checks Entered Twice	41.70	
Balance, September 30, 1937		$25,873.23

Approved.

W. W. SKINNER

REPORT OF THE AUDITING COMMITTEE

The public accountant's audit of the books of the Association of Official Agricultural Chemists, Inc., as of September 30, 1937, was ex-

amined by the Committee and found to be correct. Verification was also made of the bonds on deposit.

DAN DAHLE
B. F. ROBERTSON

Approved.

No report was given by the Committee to Cooperate with other Committees on Food Definitions.

REPORT OF THE COMMITTEE ON NECROLOGY

Since our last annual meeting the Association has suffered the loss by death of three important members. The first of these to be removed was George Leslie Bidwell, Chief of the Cattle Food Laboratory of the Food and Drug Administration, who passed away on February 20th of this year at his home in Washington at the age of 57. For the last twenty years he was a most tireless worker in the activities of this organization as Referee on Feeds and Feeding Stuffs and as a member of various committees. An eloquent sketch of his work and personality by W. B. White was published in the last August number of the *Journal.*

The second death to be recorded is that of Perry Fox Trowbridge, Director of the North Dakota Agricultural Experiment Station, who died on May 15 of this year at his home in Fargo, North Dakota, at the age of 71. He first became associated with the work of this Association in 1907, and after active participation in its various offices as referee, member of the committee on revision of methods, and vice president, officiated as president at the 1919 meeting. A fitting tribute to his work as chemist and experiment station director by C. Robert Moulton is published in the current issue of the *Journal.*

The third death to be noted is that of Orrin Bowman Winter, Research Chemist of the Michigan Agricultural Experiment Station, who died suddenly of a heart attack on the morning of October 1st of this year at his home in East Lansing, Michigan, at the age of 59. His first participation in the work of the Association was as joint author, with A. J. Patten, of a paper on the "Determination of Iron and Aluminum in the Presence of Calcium, Magnesium and Phosphoric Acid," presented at the 1927 meeting. Since 1929, as Referee on Plants, his attendance at our meetings was almost continuous. He was a tireless worker and contributed much to the Association's methods for determining the mineral constituents of plants. His sudden passing, in the midst of preparing his referee report for the present meeting, is particularly deplorable. A fuller sketch of Mr. Winter and his work for the Association will be prepared by Mr. Miller for a forthcoming issue of our *Journal* (see page iii).

I move, you, Mr. President, that we all rise as a token of respect to the memory of these lamented friends and fellow members.

C. A. Browne
H. C. Lythgoe

Approved.

REPORT OF NOMINATING COMMITTEE

The Committee on Nominations has given careful consideration to the question of recommending officers to be elected for the ensuing year. We desire to place in nomination the following:

President: H. R. Kraybill, Lafayette, Ind.
Vice-President: W. S. Frisbie, Washington, D. C.
Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.
Additional Members of the Executive Committee:
L. B. Broughton, College Park, Maryland
J. W. Sale, Washington, D. C.
G. G. Frary, Vermillion, N. D.
Post-Officio:
C. C. McDonnell, Washington, D. C.

W. H. MacIntire
L. E. Bopst
G. L. Marsh

A unanimous vote was cast for each officer nominated.

After the unanimous vote had been cast for the new president, Dr. MacIntire requested Mr. Lythgoe and Dr. Hand to conduct Dr. Kraybill to the Chair.

Mr. Lythgoe: Fellow members of the A.O.A.C. For the third time my friend MacIntire has suggested me to bring the newly elected President to the Chair and introduce him. The first time I saw this gentleman was some years ago when he was in New England. He has since journeyed to the West and I hope the New England folks will have the pleasure of getting him back. Mr. President, you will find that your duties are not very heavy but when you get through and become a past president you will cherish your connection with this Association far more than any other Association with which you have been connected, and I say that advisedly because I at one time held that position.

Dr. MacIntire: The first name on the additional list of the present Executive Committee in point of seniority is our beloved friend, C. L. Hare, of Auburn, Alabama. Dean Hare is in poor health and was unable to attend this meeting. He has asked that he be not continued as a member of the Executive Committee, and for that reason his name is not again presented. I am sure you are in accord with me in requesting that the Secretary-Treasurer be directed to send Dr. Hare a telegram expressing

appreciation of his long period of service with us, and our love and regard for him and the further hope that he will regain his health (motion carried).

The president, with the approval of the Executive Committee, renamed H. A. Lepper as Chairman of the Committee on Recommendations of Referees and appointed L. E. Bopst to fill the vacancy on the Editorial Committee of *The Journal.*

REPORT OF COMMITTEE ON RESOLUTIONS

Whereas, the Fifty-Third Annual Meeting of the Association of Official Agricultural Chemists is being concluded; and

Whereas, an unusually large attendance has profited by a comprehensive and instructive program; be it

Resolved, that we express to our president, Dr. C. C. McDonnell, our appreciation of his excellent presidental address and his able and courteous direction of our proceedings as our presiding officer.

Resolved, that we express to our past president, Dr. C. A. Browne, our appreciation of his masterly Wiley Memorial Address on "A Few Unsolved Problems of Agricultural Chemistry."

Resolved, that we extend our thanks to those members who have assisted the president by presiding over our several sectional meetings.

Resolved, that we extend our thanks to our Secretary, Dr. Skinner, to Miss Lapp, and to Mr. Frisbie for the careful thought and attention given by them to insure the interest of members and guests and the success of the meeting.

Resolved, that, through our Secretary, we extend our thanks to the management of the Raleigh Hotel for their cooperation and courtesy.

W. B. White
E. M. Bailey

Approved.

CONTRIBUTED PAPERS

LOSSES OF CHLORINE IN DIFFERENT MATERIALS WITH VARIOUS ASHING TEMPERATURES*

By T. A. Pickett (Agricultural Experiment Station, Experiment, Ga.)

The A.O.A.C. method for determining chlorine in plant materials[1] includes directions to "ignite as thoroly as possible at a temp. not exceeding dull redness." Many workers have experienced considerable difficulty with this method. Barium hydroxide, calcium carbonate, sodium carbonate, and many other materials have been added to the sample to fix the chlorine, but in many cases chlorine was lost. Tilden[2] showed that under favorable conditions a certain amount of sodium carbonate held a definite amount of chlorine and also noted that the relative quantities of carbon and chlorine materially affected the percentage loss of chlorine. No data were given concerning control of the ashing temperature.

It was believed that a study involving time and temperature of ashing might fix more definitely the conditions under which different materials might be ashed without loss of chlorine, and also determine whether the loss of chlorine varies according to the kind of sample ashed.

Chlorine was determined on the various materials shown in the table. The pineapple juice was prepared according to the method used by Tilden[3] and her collaborators.

Twenty cc. of 5 per cent sodium carbonate was added to some of the samples, which were then ashed in an electric furnace equipped with a pyrometer that checked and controlled the temperature every 15 seconds. By checking with a thermometer and a portable pyrometer to 350° C. and with the pyrometer at higher temperatures, a very slight lag in the temperature of the furnace was noted, but as this was of only a very few degrees, the error was ignored. Temperatures varying from 500° to 800° C. were used. Variations in the time of ashing were tried, but little effect on the amount of chlorine lost was noted, provided a white ash was obtained. The first and second ashings of the samples were started in a cold muffle and were of one hour duration each, with the exception of the group ashed at 800° C., which needed only one ashing to produce a white ash. After ashing, the chlorine was determined by the Volhard method, 0.05 N silver nitrate and 0.05 N potassium thiocyanate being used.

The data obtained are given in the following table:

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* Published with the approval of the Director as Paper 51, Journal Series, Georgia Agricultural Experiment Station.

[1] *Methods of Analysis*, *A.O.A.C.*, 1935, 131.
[2] *This Journal*, 11, 209 (1928).
[3] *Ibid.*, 12, 195 (1929).

Chlorine found (mg.)

	ASHING TEMPERATURE, °C.				
	500	550	600	700	800
35.72 mg. Chlorine	35.59	35.23	34.92	30.21	13.72
35.72 mg. Chlorine*	35.70		35.65	35.30	33.73
35.72 mg. Chlorine* +5 g. Sucrose	34.51		35.12	33.93	28.73
35.72 mg. Chlorine +2.5 g. Starch	31.69		31.60	28.68	
35.72 mg. Chlorine +5 g. Wheat	27.88		20.88		
35.72 mg. Chlorine* +5 g. Wheat		37.76	37.94	37.27	34.77
100 cc. Pineapple Juice*			11.82	11.65	10.50
35.46 mg. Chlorine* +100 cc. Pineapple Juice			46.32	45.53	40.11
4 g. Cotton Leaves	22.77		22.04		
4 g. Cotton Leaves*		28.15		27.25	26.27
0.1 g. Muriate of Potash*	48.50		48.49	48.55	46.00
4 g. Turnip Tops*	75.95		75.60	75.05	71.15
5 g. Tankage*	21.45		21.00	21.30	19.25
5 g. Fish Meal*	18.33		17.90	17.55	17.00
3 g. Mixed Fertilizer*	66.05		66.11	64.10	63.95

* 20 cc. of 5% Na_2CO_3 added.

As shown in the table, very little chlorine, if any, was lost at 500° and 600° C. when sodium carbonate was present. In some cases, chlorine was lost at 700° C., and at 800° C. the loss was considerable in practically every material used. The addition of extra organic matter in the form of starch increased the loss of chlorine. Wheat giving an acid ash lost an excessive amount of chlorine when sodium carbonate was not present.

SUMMARY

At temperatures up to and including 600° C. for two ashings of one hour each, the samples in this study were ashed with a negligible loss, if any, of chlorine, provided sodium carbonate was present in excess. Increasing the period of ashing made no material difference in the loss of chlorine.

Many of the materials analyzed, including muriate of potash, turnip tops, tankage, and fish meal, could be ashed safely at 650° C. when sodium carbonate was present in sufficient amounts.

None of the samples could be ashed at 800° C. without a considerable loss of chlorine.

A large amount of chlorine was lost (in some samples) even at 500° C. when there was not an excess of sodium carbonate present.

VOLATILE OIL IN MARJORAM*

By J. F. Clevenger (U. S. Food and Drug Administration, New York, N. Y.)

Most of the marjoram leaves used are grown in France and imported from Marseilles. Limited amounts of these leaves are also imported from Tunis.

During the past six years many of the importations of marjoram leaves in New York have been analyzed for the yield of volatile oil. Determinations have also been made of some of the physical and chemical characteristics of these oils.

The results reported here were obtained by the method outlined in *Methods of Analysis A.O.A.C.*, 1935, 447–449.

Volatile oil in marjoram leaves

YIELD V/W*	SP GR. 25°/25°C.	OP. ROT. 25°C.	REF. IND. 20°C.	ACID NO.	ESTER NO.
2.0	0.939	+ 9.0	1.501	3.7	13.1
1.7	0.937	+ 6.9	1.496	4.6	21 7
1.2	0.896	+13.1	1.475	3.9	33.0
1.7	0 897	+12.8	1.476	1.7	22 9
1.5	0.913	+ 9.1	1.486	3.2	25.8
1.9	0.910	+10.4	1.486	1.7	17.2
1.0	0 936	+ 9.3	1.491	4.1	20.9
2.9‡	0.931	+ 6 2	1.497	1.9	2.0
1.6	0.935	+ 8.3	1.494	4.2	15.1
2.6‡	0.935	+10 3	1.489	2.6	5.0
2.4‡	0 926	+ 9.7	1.489	1.8	14.0
1.6	0.900	+16 3	1.473	2.3	23.6
1.8	0.913	+16.8	1.475	1.1	39.0
1.5	0.945	+ 8.6	1 495	3.5	12.0
1.0	0.909	+14.0	1.478	2.0	33.7
1.3	0.950	+ 6 05	1.502	2.6	9.3
1.6	0.961	+ 4.5	1.508	2.7	6.8
1.7	0.927	+ 5.9	1.493	5.3	23.0
0.3†	0.912	+15.2	1.478	4.6	28.6

* Cc. per 100 grams of marjoram.
† Contained exhausted leaves.
‡ Grown in Tunis.

To determine the extent of loss of volatile oil in whole marjoram leaves that had stood in the laboratory, a portion of some uniformly mixed marjoram leaves was analyzed. The remaining portion of the sample was stored in the laboratory in an open shallow pan. One year later the remaining portion was analyzed. The results follow:

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 1, 2, and 3, 1937.

DATE	YIELD V/W	SP. GR. 25°/25°C.	OP. ROT. 25°C.	REF. IND. 20°C.	ACID NO.	ESTER NO.
4/29/36	1.6	0.940	+5.2°	1.503	1.87	4.67
5/29/37	1.4	0.933	+5.2°	1.503	1.65	7.9

CONCLUSIONS

1. The marjorams of commerce were found to vary considerably in the yield and physical and chemical characteristics of their volatile oil. This is probably due to differences in variety and origin of growth.

2. The volatile oils from marjoram leaves are characterized by a positive rotation. This characteristic is valuable in detecting spurious species of marjoram.

3. The loss of volatile oil from marjoram leaves that had stood one year in an open pan in the laboratory was very slight.

THE DETECTION OF VEGETABLE GUMS IN DAIRY PRODUCTS*

By Phileas A. Racicot and Carl S. Ferguson
(Massachusetts Department of Public Health, Boston, Mass.)

The use of vegetable gums in place of gelatin, particularly in cream cheese, cottage cheese, and sour cream, has been increasing within the past few years. Certain vegetable gums appear to have better emulsifying properties than gelatin, and may be used in smaller quantities. Their advocates claim that they give the resulting product a finer texture and prevent "leakage" on standing, and maintain that they are a necessity in the manufacturing process. Incidentally, they are excellent water-retaining agents, and in certain products, such as sour cream, make possible the incorporation of considerable additional *air* as well. Finally they are much more difficult to detect than is gelatin.

The discovery of a quantity of vegetable gum in a cheese-manufacturing establishment, together with the lack of a suitable method of detection, led the writers to undertake the study presented in this paper.

The A.O.A.C. method is cumbersome, not entirely accurate, and does not differentiate between the various gums. The method developed by the writers is submitted with the knowledge that it is limited in its application. They feel, however, that it is simpler than the A.O.A.C. method and that it is accurate in detecting the presence of vegetable gums as well as the identification of locust bean gum.

The method is based on the fact that practically all of the various gums contain a substance of carbohydrate nature, which, on hydrolysis, yields

* Presented by H. C. Lythgoe at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 1, 2, and 3, 1937.

a sugar, the presence of which is ascertained by well-known accepted tests. The method requires the isolation in pure form of a portion of this carbohydrate material separated from other carbohydrates naturally present in the sample as well as from the milk proteins and decomposition products. The tests are made directly on this isolated material.

Trichloracetic acid solution is used for separation of the milk protein from the gum. Of the gums studied, a quantity sufficient for detection of locust bean gum, gum tragacanth, gum arabic, and agar passes into the filtrate. The gum is precipitated from the filtrate by alcohol. The character of the precipitate gives some indication of the identity of the gum present.

After precipitation, the gum is washed thoroughly to remove all lactose originally present. This is best accomplished by centrifuging and decanting. After being washed, the precipitate is tested: first, to make sure it is free from reducing substances and protein matter; and second, to show that it does contain carbohydrate material. It is then subjected to hydrolysis, and again tested for the presence of reducing substances. If reducing substances are now found, the presence of a vegetable gum is established. In order to prove the identity of the gum a further test is made with phenylhydrazine, and the osazones thus obtained are examined microscopically.

THE METHOD

Preliminary Test

To 10 grams of the sample add 10 cc. of distilled water, mix thoroughly, and add 5 cc. of 20% trichloracetic acid solution. Shake for 1 minute and filter. To 1 volume of the filtrate in a test tube, add 2 volumes of 95% alcohol and mix. If the resulting mixture fails to show a distinct precipitate or turbidity after standing 30 minutes, the test is negative. The appearance of a stringy or flocculent precipitate indicates the presence of locust bean gum or gum tragacanth. A turbidity settling out as a fine granular precipitate indicates gum arabic. A turbidity that persists even after standing overnight is due to decomposition or fermentation products present in the sample and not to the presence of gum.

If the preliminary test indicates the presence of vegetable gum, proceed with the confirmatory test as follows:

Confirmatory Test

To 100 grams of the sample add 100 cc. of water, and mix well; add 50 cc. of 20% trichloracetic acid, shake for 1 minute, and filter. To the entire filtrate add 2 volumes of 95% alcohol. Mix thoroughly and let stand overnight, preferably in a tall cylinder or graduate.

Decant the supernatant liquor as completely as possible without loss of precipitate so that not more than 50 cc. remains. Mix the precipitate with the remainder of the filtrate and pour into a 50 cc. centrifuge tube. Centrifuge 5–10 minutes, or until the supernatant liquor can be poured off without disturbing the precipitate. Wash the precipitate 6 times with 50 cc. of 75% alcohol, shaking well, centrifuging, and draining the supernatant liquor as completely as possible each time to remove all traces of lactose.

Add to the entire precipitate 15 cc. of distilled water and mix. Note whether the precipitate is soluble or whether it forms merely a suspension in water. If it dis-

solves, gum arabic is indicated and may be identified by U. S. P. tests on this solution. To prove the absence of lactose, conduct Benedict's qualitative test upon this mixture, using 8 drops of the mixture and 5 cc. of Benedict's solution. Conduct the Biuret test on 1 cc. of the mixture to prove the absence of protein. Conduct Molisch's carbohydrate test on 1 cc. of the mixture to prove that the precipitate is a carbohydrate.

Hydrolyze 10 cc. of the mixture with 10 cc. of HCl (1 vol. conc. HCl+2 vols. water), boiling gently 2–3 minutes. Cool, and neutralize exactly to phenolphthalein with NaOH, using 50% NaOH at first and finally adjusting the reaction to exact neutrality with 0.1 *N* NaOH or 0.1 *N* HCl. Cool, and add 1 or 2 grams of decolorizing carbon; shake and filter.

Conduct Benedict's qualitative test upon 8 drops of this filtrate, and let stand overnight if there seems to be no immediate reduction. Comparison should be made with Benedict's test before hydrolysis.

A negative Biuret test for protein plus a positive Molisch carbohydrate test and a negative Benedict test before hydrolysis, followed by a positive Benedict test after hydrolysis, are proof that a vegetable gum is present.

To 10 cc. of the neutralized solution, add 1 gram of phenylhydrazine hydrochloride and 1.5 grams of sodium acetate. Mix thoroughly and place in a boiling water bath for 2 hours; allow to cool and note presence of osazones. Examine under the microscope, comparing if necessary with osazones of known identity. Glucosazones are characteristic of locust bean gum; tragacanth gives flat pale yellow osazones resembling maltosazones; and gum arabic gives very small burr-like crystals.

DISCUSSION

The writers observed that Irish moss is precipitated by proteins in an acid solution. Since gums are separated from dairy products by trichloracetic acid in the procedure described above, this method is impractical for the detection and identification of Irish moss.

The writers also observed that karaya gum is not precipitated by the addition of alcohol to the trichloracetic acid filtrate and, therefore, can not be detected by this method.

Since locust bean gum has been accepted by the manufacturers of cheese as that which best serves their purpose and is most used at the present time, the method being especially accurate in the detection and identification of locust bean gum is of real value to the food chemist. The preliminary test by which the presence or absence of vegetable gum is determined and which, to a certain degree, identifies some of the gums can be made in 30 minutes. Ten samples can be tested in one hour. The confirmatory test requires standing overnight, several washings in a centrifuge, and tests before and after hydrolysis, all of which consume time, but which can be performed in a day. The confirmatory test is valuable for prosecution purposes, but is not necessary in ordinary routine work.

DIRECT DETERMINATION OF AVAILABLE P_2O_5 CONTENT OF FERTILIZERS*

By W. H. MacIntire, W. M. Shaw, and L. J. Hardin (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

The official method for determination of "available" P_2O_5 (1) is based upon the procedure of Fresenius, Neubauer, and Luck (4). Considered in 1880 by the meeting (3) that presaged the formation of the Association of Official Agricultural Chemists, the method was adopted by the Association in 1884 (2). Proposed and intended solely for the evaluation of acidic types of fertilizers, the method has served well its intended utility. The troublesome factor of inexactness of reaction of the citrate solvent was eliminated as a result of the studies conducted by Robinson (14), (15), (16), (17).

During recent years, however, ammoniation and incorporation of dolomite have resulted in types of fertilizers that contain basic compounds that vitiate the solvent capacity of the official citrate extractant. This fact has been demonstrated effectively by the contributions that have appeared in *This Journal*, 1931–1937, and by contemporary studies (5–13 inc.), (18), (19). In his 1931 report, the Associate Referee on Phosphoric Acid stressed the need for study and revision to make the official method suitable for analysis of processed superphosphates (18). Since then it has been shown that heat of reaction during curing materially increases the citrate-insoluble P_2O_5 content of ammoniated superphosphates and that formation of fluorapatite takes place in processed superphosphates during curing and analysis (13).

It seemed probable that a solvent could be developed to effect the same removal of P_2O_5 that is now accomplished jointly by aqueous washing and citrate digestion. Such single solvent should (a) admit of direct precipitation of its P_2O_5 content; (b) its pH during analytical technic should be near-constant; (c) it should induce no P_2O_5 retrogradation during analysis and (d) its capacity to dissolve should not be materially lessened by common ion effect. Studies leading to these objectives were conducted upon phosphatic materials of widely variant type, composition, and concentration.

The step of leaching water-soluble P_2O_5 was simulated by use of a cold 1 *M* solution of ammonium nitrate to effect substantial and even complete removal of both "available" phosphate and calcium sulfate contents. Further removals, simulating those by the citrate digestion of water-insoluble residues, were effected by boiling digestion of the leached residues with the nitrate solution. Leachates and digestates were combined

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* A collaborative study under the auspices of the Tennessee Valley Authority, Department of Chemical Engineering. Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 1, 2, and 3, 1937.

to volume, and the P_2O_5 content of the solution was determined. This technic was found adequate for many acidulated materials, but it was found necessary to fortify the 1 *M* ammonium nitrate solution with 0.05 *M* ammonium citrate to obviate precipitative tendencies encountered with superphosphates made from certain types of rock phosphate.

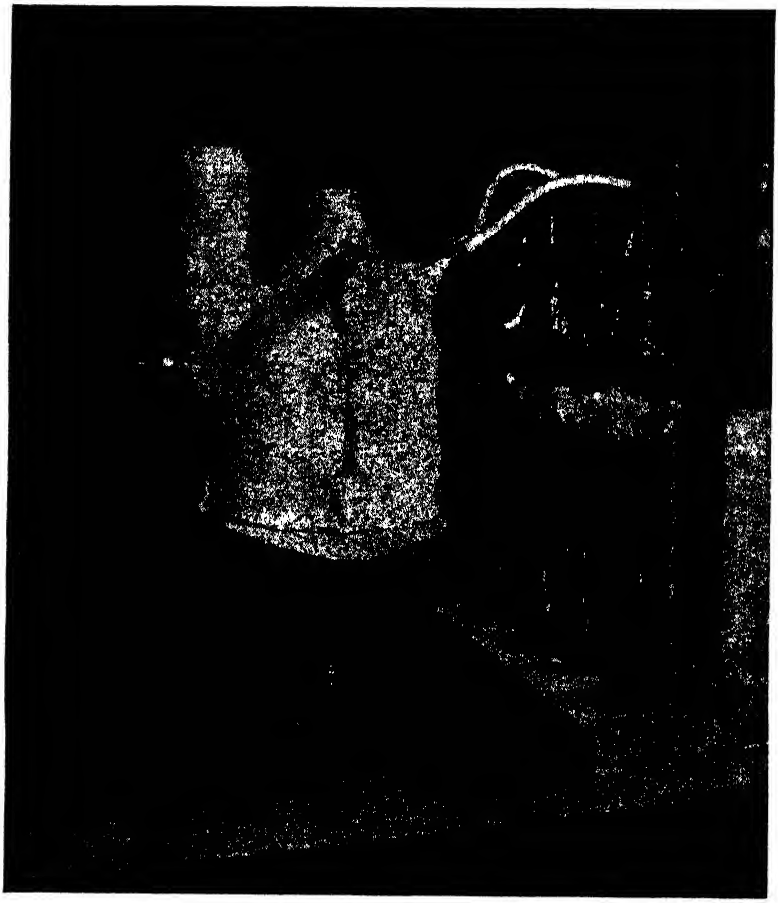

FIG. 1.—INSULATED HOMEMADE BAFFLED COPPER STILL, CONNECTED WITH FOUR FERTILIZER FLASKS FOR STEAM DIGESTION WITH "SOLVENT"

The prior leaching with the ultimate solvent was found to remove substantial proportions of basic phosphates, and sulfates almost completely, from ammoniated fertilizers and from all other orthophosphatic materials, except raw rock. When the basicity of the fertilizer was such as to render basic the leachates from the acidic solvent, the leachates were brought back to the initial *p*H of the solvent, to preclude precipitations therein. In the boiling digestion of the basic residues not removed by the

leachates of the dual ammonium salt solution, it was necessary to expel engendered ammonia as rapidly as formed to prevent rise in pH and resultant decrease in solvent capacity. This removal of ammonia was accomplished by a vigorous current of steam. The mean of the amounts of P_2O_5 dissolved by the ammonia-purged digestions of 6 basic fertilizers was 2.2 times the corresponding mean of the amounts obtained by refluxed boiling digestions.

The removal of ammonia by a balanced influx and outgo of steam can be considered as involving base exchange reactions and ultimate production of solutions of monoammonium phosphate, as indicated by the equations:

$$CaHPO_4 + 2NH_4NO_3 \rightarrow Ca(NO_3)_2 + (NH_4)_2HPO_4$$
$$\downarrow$$
$$NH_4H_2PO_4 \rightarrow NH_3 \uparrow$$

$$Ca_3(PO_4)_2 + 6NH_4NO_3 \rightarrow 3Ca(NO_3)_2 + 2(NH_4)_2HPO_4 + 2NH_3 \uparrow$$
$$\downarrow$$
$$2NH_4H_2PO_4 \rightarrow 2NH_3 \uparrow$$

Extensive studies were carried out to determine optimal composition and concentration of the nitrate solution, its capacity to dissolve phosphatic fertilizers in direct digestions and when used for both prior washing and subsequent steam digestion of leached residues; proportions of components removed by the prior washing; speed of the solvent action; and additional factors of charge, particle size, constancy of pH, period of digestion, common ion effect, P_2O_5 transitions during analysis, differential solubilities of different types and mixtures of engendered basic phosphates, and dissolving capacity of the ammonium nitrate-citrate solution for calcined and fused rock phosphates, slags, bones, and raw rock alone and as fertilizer supplements.

From those studies was developed the proposed procedure, which is applicable to all forms of commercial fertilizers. For brevity, the ultimate solution used for prior washing and subsequent digestion—1 M ammonium nitrate—0.05 M ammonium citrate, pH 4.2—will be designated as "solvent." The citrate concentration of "solvent" is only 1/9 of that of the "official" solution and admits of direct cold P_2O_5 precipitations, which cannot be made from the official citrate solution.

The proposed method follows:

DIRECT DETERMINATION OF P_2O_5 AVAILABILITY IN FERTILIZERS BY CITRATED AMMONIUM NITRATE

REAGENT

Citrated ammonium nitrate "solvent."—Prepare a stock solution, each liter to contain 80 grams of P_2O_5-free NH_4NO_3, 50 ml. of 1 M citric acid, and 75 ml. of 1 M NH_4OH. This dual salt solution (1 M nitrate—0.05 M citrate) should have a pH of 4.2.

PROCEDURE

(1) *Mixed fertilizers.*—Weigh 1.0 gram into a small porcelain dish, and wet carefully with 5 ml. of solvent; triturate, and transfer onto a 9 cm. gravity filter, and leach into a 150 ml. beaker with several 10 ml. portions of the cold "solvent." Add 0.5 ml. of 0.04% solution of bromcresol green to the leachate. If a blue or bluish green color develops add, dropwise, sufficient nitric acid (1+9) to produce a change to light green. Continue leaching to a final volume of 100 ml., and maintain the color as before. Transfer the filter to the 250 ml. "fertilizer" flask, A, and add 100 ml. of the "solvent." Stopper the flask tightly and disintegrate the filter by vigorous agitation. Rinse the stopper and the neck of the flask with a small amount of distilled water. Adjust outlet B to trap E and bring the suspension to boiling by means

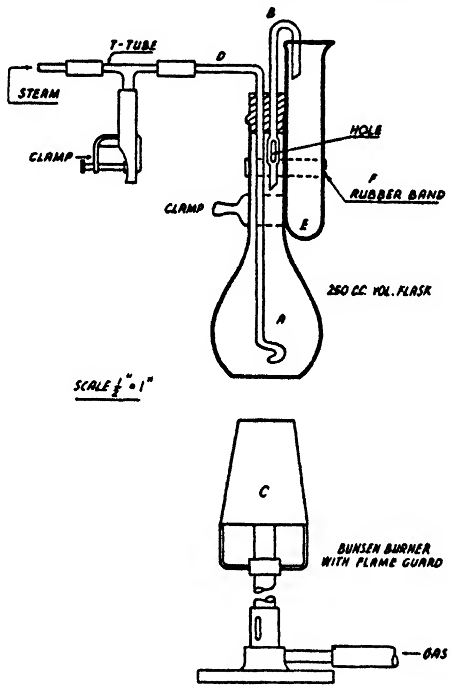

FIG. 2.—DETAIL OF FERTILIZER FLASK, INLET, OUTLET, AND GUARD USED IN DIGESTION WITH "SOLVENT"

of a small Bunsen burner, C, provided with a flame guard. Connect tube D with steam generator and pass steam through the suspension in flask A for 30 minutes, regulating the flame to maintain a volume of approximately 100 ml. Remove the flame and disconnect the flask from the steam generator; wash both outside and inside of inlet tube and the liquid from the safety tube, E, back into the digestion flask, A. Place an inverted beaker over the neck of the flask and cool under the tap; then add the prior 100 ml. leachate, make to volume, and mix. Filter a sufficient quantity of the solution through an 18.5 cm. dry fluted filter, and discard the first 25–30 ml. Use a 25 ml. (0.1 g. equivalent) aliquot and precipitate ammonium phosphomolybdate as in "official" method.

(2) *Standard superphosphates.*—Proceed as for "mixed fertilizers," but reduce the steam digestion period to 15 minutes.

(3) *Triple superphosphates.*—Proceed as for "standard superphosphates," but use a 0.5 gram charge.

(4) *Calcined and fused rock phosphates.*—Use a 0.5 gram charge and proceed as for "mixed fertilizers."

(5) *Bone meal.*—Proceed as for "mixed fertilizers."

(6) *Basic slag.*—Proceed as for "mixed fertilizers."

(7) *Metaphosphates.*—Proceed as for "calcined and fused rock phosphates."

TABLE 1.—*Available P_2O_5 contents of superphosphates and mixed fertilizers, as determined by the official and the direct citrated ammonium nitrate procedures*

CODE NO.[a]	PHOSPHATIC MATERIAL	TOTAL P_2O_5	AVAILABLE P_2O_5 BY		DIFFERENCE BY AMMONIUM NITRATE
			OFFICIAL PROCEDURE	CITRATED AMMONIUM NITRATE[d]	
		per cent	per cent	per cent	per cent
1960	Standard superphosphate[b]	20.80	20.07	19.70	−0.37
1066	Standard superphosphate[b]	20 00	19.95	20.13	+0.18
1087	Standard superphosphate[b]	21.60	20 90	20.65	−0.25
1378	Standard superphosphate[b]	20.50	16 90	16.75	−0.15
1522	Mixed fertilizer	20.86	20.74	20.60	−0.14
1526	Mixed fertilizer	16.90	16 78	16.40	−0.38
1337	Triple superphosphate[c]	46.70	46.52	46.50	−0.02
1338	Triple superphosphate[c]	45.00	44.95	44.80	−0.15
1368	Triple superphosphate[c]	47 80	44.76	44 65	−0.11
1415	Triple superphosphate[c]	48.80	46 24	46.80	+0.56
—	Triple superphoaphate[c] (W.D. 31847)	48.65	47.05	47.40	+0.35
1518	Ammoniated triple superphosphate	47.60	45.80	47.00	+1.20
1104	Base goods	13.83	12 83	12.52[e]	−0.31
1105	Base goods	9.70	6 70	6.98[e]	+0 28
1508	Mixed fertilizer	9.40	8.88	8.75[e]	−0.13
1522	Mixed fertilizer	20.86	20.74	20.63[e]	−0 11
1523	Mixed fertilizer	10.60	10.53	10 30[e]	−0.23
1524	Mixed fertilizer	10.80	10.65	10 35[e]	−0.30
1525	Mixed fertilizer	9.10	8.92	8.85[e]	−0.07
1526	Mixed fertilizer	16.90	16.78	16.35[e]	−0.43
1527	Mixed fertilizer	8.50	8.44	8.45[e]	+0.01
R	Mixed fertilizer	17.80	15.60	16.38[e]	+0 78
—	Tricalcium phosphate, c.p.	39.60	16.90	38.4	+21.50

[a] Numbers are those of Bureau of Chemistry and Soils.
[b] 1 gram charges of standard products.
[c] 0.5 gram charges of the concentrated materials.
[d] 1*M* ammonium nitrate—0.05*M* ammonium citrate solution, *p*H 4.2; preliminary leaching and 15 minute boiling digestion.
[e] Same as (d) except boiling digestion period was 30 minutes.

Hydrolyze a 10 ml. aliquot by digestion with 5 ml. of concentrated HNO_3 at boiling point for 15 minutes; cool, nearly neutralize, and proceed as in official method. (Prescribed technic calls for charge of 200-mesh.)

(8) *Rock phosphates.*—Introduce, directly into flask, A, a 1 gram charge and 100 ml. of "solvent," and proceed to digest as in "mixed fertilizers." Cool, and make to volume. Filter, and refilter if necessary, until the filtrate is clear. Use a 25 ml. aliquot and proceed as in official method.

COMPARATIVE RESULTS BY OFFICIAL AND PROPOSED PROCEDURES

For superphosphates and acidic mixed fertilizers, the official and proposed procedures gave generally concordant results, the ultimate comparison being substantially the respective solvent capacities of the "citrate" and "solvent" digestions upon residues of undecomposed rock. "Solvent" gave higher P_2O_5 values, however, for ammoniated superphosphates, separate charges of precipitated tricalcium phosphates and hydroxy apatite, calcined rock, fused rock, bone meals, and basic slags. For raw rocks, results by "solvent" were lower than those by neutral citrate extractions; but when the 1.09 sp. gr. citrate solution and "solvent" were used at the same *p*H value of 4.2, the solvent capacity of "solvent" was only ⅓ of that of the citrate for raw rock.

As indicative of values obtained for various acidic and ammoniated

TABLE 2.—*Available P_2O_5 content of ammoniated and basic superphosphates as determined by the official and the direct citrated ammonium nitrate procedures*

CODE NO[a]	TREATMENT: AMMONIATED WITH NH_3	TREATMENT: ADDITIONAL	TOTAL P_2O_5	AVAILABLE P_2O_5 BY: OFFICIAL PROCEDURE	AVAILABLE P_2O_5 BY: CITRATED AMMONIUM NITRATE[bc]	DIFFERENCE BY NITRATE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1169	2.5	none	20.60	18.90	18.80	−0.10
1175	5.4	none	19.40	18.00	17.50	−0.50
1168	8.7	none	19.40	13.80	13.30	−0.50
Ma	4.9	none	19.30	14.30	16.80	+2.50
580	8.0	none	24.60	19.50	19.05	−0.45
Me	0	Dolomite, 10	19.10	18.10	18.50	+0.40
Mf	0	Dolomite, 30	16.05	15.10	15.60	+0.50
Mb	4.4	Dolomite, 10	18.45	13.90	16.50	+2.60
Mc	8.7	none	40.00	37.15	40.00	+2.85
576	12.4	none	43.70	39.70	40.65	+0.95
1518	6.0	none	47.60	45.80	47.00	+1.20
571	0	Limestone, 30	44.70	34.40	36.15	+1.75
573	0	Dolomite, 30	41.40	37.80	38.60	+0.80
Md	7.9	Dolomite, 10	37.50	34.09	36.30	+2.21

[a] Numerals are those of Bureau of Chemistry and Soils; letters those of samples supplied by F. G. Keenen of DuPont Ammonia Experiment Station.
[b] 1 gram charges of standard products; 0.5 gram charges of triple superphosphate products.
[c] 1 *M* ammonium nitrate—0.05 *M* citrate solution; *p*H 4.2; preliminary leaching and 30 minute period of boiling digestion.

TABLE 3.—*Comparison of available P_2O_5 in ammoniated superphosphates by the official and the direct citrated ammonium nitrate procedures*[a]

CODE	PHOSPHATE FERTILIZER		TOTAL P_2O_5	AVAILABLE P_2O_5		
	TYPE	UREA AMM. LIQUOR ADDITIONS		OFFICIAL PROCEDURE	CITRATED AMMONIUM NITRATE	DIFFERENCE BY NITRATE
		lbs. per ton	*per cent*	*per cent*	*per cent*	*per cent*
1-A	Superphosphate[b]	200	19.0	16.35	16.93	+0.58
1-B	Superphosphate[b]	200	19.0	12.40	14.00	+1.60
2-A	Superphosphate[b]	170	19.5	17.30	17.55	+0.25
2-B	Superphosphate[b]	170	19.5	14.20	15.45	+1.25
3-A	Superphosphate[b]	140	20.0	19.50	19.10	−0.40
3-B	Superphosphate[b]	140	20.0	18.05	18.78	+0.73
4-A	Mixed fertilizer[c]	90	9.5	8.86	8.83	−0.03
4-B	Mixed fertilizer[c]	90	9.5	7.60	8.40	+0 80
5-A	Mixed fertilizer[c]	120	9.5	7.26	8 48	+1.22
5-B	Mixed fertilizer[c]	120	9.5	7 00	7.85	+0.85
6-A	Mixed fertilizer[c]	90	13.75	13.45	13.35	−0.10
6-B	Mixed fertilizer[c]	90	13.75	11.75	12.23	+0.48

[a] All values obtained by use of 1 gram charges.
[b] All of the superphosphates were aged 38 weeks; suffix A connotes aging at 43° C; suffix B connotes aging at 54° C.
[c] All of mixed fertilizers were aged for 42 weeks; suffix A connotes aging at 43° C; suffix B connotes aging at 54° C.

superphosphates and mixed fertilizers, by the official and proposed procedures, three tables of data are submitted.

The proposed procedure is considered by the writers to have certain advantages, namely:

(1) Simplicity, rapidity, reagent economy, and accuracy consonant with that of the official method for superphosphates and acidic fertilizers.

(2) A single, easily made, and inexpensive solvent.

(3) Maintenance of near-constancy in *p*H of "solvent" throughout digestions.

(4) One P_2O_5 determination for each fertilizer.

(5) Applicability, with minor variations, to all forms of phosphatic fertilizers.

(6) Minimal interference of common-ion effect upon solubility of phosphates in the analytical systems.

(7) Marked reduction in the error introduced by hydrolysis and by the formation of fluorapatite, which occur during "official" aqueous leaching and citrate digestion of ammoniated and limed superphosphates.

(8) Elimination of difficulty in obtaining clear filtrates.

(9) Higher values, greater accuracy and uniformity in values obtained

for tricalcium phosphates, as such, and as components of ammoniated superphosphates.

(10) Values more in accord with those known to reside in calcined rocks and fused rocks.

(11) Less variation incident to particle size of both raw and calcined rock phosphates.

(12) Less enhancement in "availability" values for fertilizers carrying additions of raw rock.

(13) No vitiation of results by the inclusion or the absence of macerated filter.

(14) Elimination of noxious fumes incident to digestion of insoluble residues.

CONCLUSIONS

Since "solvent" (a) dissolves completely both dicalcium and tricalcium phosphates of charges greater than amounts to be encountered in prescribed charges of fertilizers, (b) dissolves hydroxyapatite as readily as it does tricalcium phosphates, (c) is maintained at *p*H that obviates formation of fluorapatite during digestion, (d) dissolves fluorapatite sparingly in presence of other basic phosphates, (f) possesses a higher solvent capacity for calcined rocks, fused rock, bones and basic slags that reflect their known values, (g) consistently shows somewhat lower values for raw rock, and (h) gives values concordant with those obtained by use of citrate for acidic types of fertilizers, it is concluded that the proposed procedure gives readily duplicable results that afford equitable evaluations for all phosphatic fertilizers.

The present paper is offered as a preliminary report of the studies conducted in the evolution of the proposed method.

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INTENSIFIED STUDY OF THE USE OF THE REFRACTOMETER AS A CHECK IN BEER ANALYSES*

By E. A. Siebel and Arthur E. Kott (E. A. Siebel & Co., 8 South Dearborn St., Chicago, Ill.)

The refractive index and the specific gravity of beer vary with the amounts of dissolved substances. The total of non-volatile substances in beer, calculated as sucrose from the specific gravity, is called the extract. The amounts of alcohol and of extract can be calculated from the refractive index of the beer or the scale reading in the refractometer, and the specific gravity. These calculations are especially useful as a check on the standard distillation method, since the readings are made on the beer itself.

PROPOSED FORMULAS

Barth,[1] basing his work partly on that of Tornoe[2] and of Ackermann[3] developed formulas for the percentage of alcohol, A, and of extract, E, by weight, in terms of the difference between the refractive index of the beer and of distilled water, r, and the difference between the specific gravity of the beer and of water, n, at 17.5°C.:

$$A_w = 759.8r - 292.3n. \tag{1}$$
$$E_w = 336.6r + 130.3n. \tag{2}$$

Race[4] found that for English beers of more than 4.5 per cent alcohol the following formulas gave better agreement:

$$A = 778r - 290n. \tag{3}$$
$$E = 350r + 130n. \tag{4}$$

Lehmann and Gerum,[5] from a study of a large number of German beers, developed formulas for the alcohol and extract as grams per 100 cc., from the difference, R, between refractometer scale readings of beer and water, at 17.5°C., and N, a function of the specific gravity:

$$N = 1000\ (\text{Sp. gr.} - 1). \tag{5}$$

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 1, 2, and 3, 1937.

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$$A = \frac{2}{7}(R - N). \tag{6}$$

$$E = \frac{0.9}{7}(R + N) + k. \tag{7}$$

An immersion refractometer, for which the scale reading at 17.5°C. was 15.0, was used. The specific gravity was determined at 15/15°C., and the value taken for k varied with the alcohol:

A	1	2	3	4	5
k	0.04	0.05	0.08	0.07	0.04.

Berglund, Emlington, and Rasmussen[1] worked out formulas for Scandinavian beers, giving alcohol and extract by weight:

$$A_w = 0.2691R - 0.2774N + 0.323 \tag{8}$$

$$E_w = 0.1179R + 0.1298N + 0.251. \tag{9}$$

The specific gravity was determined at 20/20°C., and the refractometer reading made at 20°C.

Ackermann and Steinmann[2] and Ackermann and Toggenburg[3] proposed a different calculation, involving refractometer readings on beer and distillate (made up to original volume) without the specific gravity of the beer. Alcohol as grams per 100 cc. can be obtained from the reading on the distillate, and for extract as grams per 100 cc. they gave the formula—

$$E = 0.25705(R_b - R_d). \tag{10}$$

The readings were made at 17.5°C. This formula is given also in the *Methods of Analysis*.[4]

TEST OF PROPOSED FORMULAS

Results of the analyses of a number of American beers were used to test the accuracy of the formulas given, and of new formulas. Twenty beer samples, from twelve breweries, were analyzed in duplicate by different analysts for specific gravity, refractive index, alcohol, and extract.

The alcohol and extract determinations were made according to the official methods of analysis of the A.O.A.C. For the alcohol a determination with pycnometer was made of the specific gravity of a distillate from 100 cc. of the beer. The extract was determined by a calculation in which the effect of the alcohol on the specific gravity, found from the distillate, was added to the specific gravity of the beer. The Plato degree (according to the Plato table) of the resulting specific gravity was multiplied by that specific gravity to obtain the grams of extract per 100 cc. of beer. The specific gravity of the beer was determined at 20/20°C. in air with a

[1] *Woch. Brau*, 51, 233 (1934).
[2] *Z. ges. Brauw.*, 28, 259 (1905).
[3] *Ibid.*, 29, 145 (1906).
[4] *Methods of Analysis, A.O.A.C.*, 1935, p. 149.

pycnometer. The refractometer reading was made with an immersion refractometer at 20°C., and the refractive index relative to air was calculated from the reading.

The analytical data are given in Table 1. In Table 2 are shown the values of alcohol and extract calculated by the three most accurate of the proposed formulas. The formulas of Race gave considerably greater deviations, and the formula of Ackermann was known from previous tests, in which the refractometer reading was made on the distillate also, to give inconsistent results. In the case of formulas expressed in percentage

TABLE 1.—*Analytical data*

NO.	BREWERY	SPECIFIC GRAVITY OF BEER, 20/20°C.	SPECIFIC GRAVITY OF DISTILLATE, 20/20°C.	DIFF. BETWEEN REFRACTOMETER READINGS OF BEER AND WATER, 20°C.	REFRACTIVE INDEX OF BEER, 20°C.	ALCOHOL	EXTRACT
						g./100 cc	*g /100 cc*
1	A	1.01210	0.99341	24.35	1.34238	3.60	4.85
2	B	1.01568	0.99265	29.75	1.34442	4.05	5.98
3	C	1.00970	0.99451	19 80	1.34064	2.98	3.93
4	C	1.00914	0.99479	18 70	1.34022	2.82	3.70
5	D	1.00556	0.99659	11 60	1.33749	1.82	2.30
6	E	1 01342	0.99372	24.95	1.34260	3.42	5.08
7	E	1.00955	0.99419	20 35	1.34085	3.16	3.98
8	C	1.00974	0.99482	19.20	1.34041	2.80	3.87
9	F	1.01414	0.99342	26.75	1.34329	3.59	5.37
10	F	1.01856	0.99379	30.10	1.34455	3.38	6.43
11	G	1.01162	0.99379	23.10	1.34190	3.38	4.62
12	H	1.01583	0 99399	26.95	1.34336	3.27	5.66
13	I	1.01732	0.99301	30.20	1 34459	3.83	6.31
14	B	1.01658	0 99410	27.55	1.34359	3.21	5.83
15	E	1.01254	0.99364	24.35	1.34238	3.46	4.89
16	G	1.01160	0.99388	22.90	1.34182	3.34	4.59
17	J	1.01543	0.99437	26.00	1.34300	3.06	5.46
18	K	1.01430	0.99412	25.35	1.34276	3.20	5.23
19	L	1.01281	0.99345	24.75	1.34253	3 57	5 01
20	B	1.01816	0.99129	35.20	1.34648	4.85	6.97

by weight (grams per 100 grams), the values obtained were multiplied by the specific gravity of the beer, so that all results might be on a uniform basis of grams per 100 cc. The error caused by the use of specific gravity at 20/20°C. and the refractometer readings at 20°C., instead of values for 15°C. or 17.5°C., as called for in some of the formulas, is negligible, since the effect of temperature variation on the ratios and differences involved here is very slight.

Deviations as large as 0.07–0.18 per cent, with average deviations of 0.04–0.09 per cent, are much greater than the experimental error. The precision of the refractometer reading is less than that of the specific

TABLE 2.—*Results with proposed formulas*

(GRAMS PER 100 CC.)

	BARTH				LEHMANN AND GERUM				BERGLUND ET. AL.			
NO.	ALCOHOL	DEVIATION: ALCOHOL	EXTRACT	DEVIATION: EXTRACT	ALCOHOL	DEVIATION ALCOHOL	EXTRACT	DEVIATION: EXTRACT	ALCOHOL	DEVIATION: ALCOHOL	EXTRACT	DEVIATION: EXTRACT
1	3.63	+0.03	4.80	−0.05	3.50	−0.10	4.76	−0.09	3.55	−0.05	4.75	−0.10
2	4.16	+0.11	5.98	0	4.02	−0.03	5.91	−0.07	4.03	−0.02	5.89	−0.09
3	3.00	+0.02	3.87	−0.06	2.89	−0.09	3.87	−0.06	2.99	+0.01	3.88	−0.05
4	2.85	+0.03	3.65	−0.05	2.73	−0.09	3.65	−0.05	2.84	+0.02	3.68	−0.02
5	1.80	−0.02	2.24	−0.06	1.73	−0.09	2.26	−0.04	1.91	+0.09	2.35	+0.05
6	3.42	0	5.05	−0.03	3.29	−0.13	5.02	−0.06	3.35	−0.07	5.00	−0.08
7	3.19	+0.03	3.93	−0.05	3.09	−0.07	3.93	−0.05	3.18	+0.02	3.93	−0.05
8	2.82	+0.02	3.81	−0.06	2.70	−0.10	3.79	−0.06	2.83	+0.03	3.81	−0.06
9	3.75	+0.16	5.38	+0.01	3.60	+0.01	5.33	−0.04	3.65	+0.06	5.31	−0.06
10	3.41	+0.03	6.43	0	3.30	−0.08	6.34	−0.09	3.33	+0.05	6.33	−0.10
11	3.41	+0.03	4.56	−0.06	3.28	−0.10	4.54	−0.08	3.36	−0.02	4.53	−0.09
12	3.30	+0.02	5.63	−0.03	3.18	−0.09	5.58	−0.08	3.23	−0.04	5.57	−0.09
13	3.81	−0.02	6.27	−0.04	3.68	−0.15	6.18	−0.13	3.71	−0.08	6.16	−0.15
14	3.25	+0.04	5.82	−0.01	3.13	−0.08	5.75	−0.08	3.18	−0.03	5.74	−0.09
15	3.51	+0.05	4.85	−0.04	3.37	−0.09	4.82	−0.07	3.43	−0.03	4.81	−0.08
16	3.36	+0.02	4.53	−0.06	3.23	−0.11	4.52	−0.07	3.30	−0.04	4.51	−0.08
17	3.15	+0.09	5.46	0	3.02	−0.04	5.41	−0.05	3.09	+0.03	5.40	−0.06
18	3.29	+0.09	5.22	−0.01	3.16	−0.04	5.18	−0.05	3.22	+0.02	5.17	−0.06
19	3.55	+0.02	4.94	−0.07	3.41	−0.16	4.91	−0.10	3.47	−0.10	4.89	−0.12
20	5.03	+0.18	7.03	+0.06	4.87	+0.02	6.91	−0.06	4.84	−0.01	6.88	−0.09
Average deviation		0.05		0.04		0.09		0.07		0.04		0.08
Greatest deviation		0.18		0.07		0.16		0.13		0.10		0.15

gravity determination, but still an error of 0.1 in the refractometer scale reading (it need not be greater) corresponds to less than 0.03 per cent alcohol. It is evident that some factor not included in the proposed formulas has an influence on the results.

DERIVATION OF FORMULAS

The effects of all the dissolved substances in beer on the refractometer reading and on the specific gravity function may be represented as the sum in each case of the separate effects of alcohol and of extract:

$$R = aA + bE. \tag{11}$$

$$N = -cA + dE. \tag{12}$$

The coefficients a, b, c, and d represent the effects on the refractometer reading and on the specific gravity function of the alcohol and of the extract, divided in each case by the amount present, in grams per 100 cc. From these equations the following are obtained:

$$A = \frac{R}{a + \frac{b}{d}c} - \frac{N}{\frac{d}{b}a + c}. \tag{13}$$

$$E = \frac{R}{b + \frac{a}{c}d} + \frac{N}{\frac{c}{a}b + d}. \tag{14}$$

These expressions have the same form as the formulas that have been discussed.

TABLE 3.—*Specific gravity and refraction coefficients*

ALCOHOL A	ALC EFFECT ON REFRACTION ÷GRAMS ALC. PER 100 CC. R/A OR a	ALC. EFFECT ON SP. GR. ÷GRAMS ALC. PER 100 CC. N/A OR c	SUM OF TWO ALCOHOL COEFFICIENTS $a+c$	EXTRACT E	EXT EFFECT ON REFRACTION ÷GRAMS EXT. PER 100 CC. R/E OR b	EXT. EFFECT ON SP. GR. ÷GRAMS EXT PER 100 CC. N/E OR d
g./100 cc.				*g./100 cc.*		
0.50				0.50	3.69	3.90
1.00	1.49	1.89	3.38	1.00	3.71	3.89
1.50	1.55	1.88	3.43	1.50	3.70	3.88
2.00	1.59	1.87	3.46	2.00	3.71	3.87
2.50	1.61	1.86	3.47	2.50	3.71	3.87
3.00	1.63	1.84	3.47	3.00	3.71	3.87
3.50	1.65	1.83	3.48	3.50	3.71	3.87
4.00	1.66	1.82	3.48	4.00	3.72	3.86
4.50	1.67	1.80	3.47	4.50	3.72	3.86
5.00	1.68	1.79	3.47	5.00	3.72	3.86
5.50	1.69	1.78	3.47	5.50	3.72	3.86
6.00	1.70	1.76	3.46	6.00	3 72	3.86
6.50	1.71	1.75	3.46	6.50	3.73	3.86
7.00	1.72	1.74	3.46	7.00	3.74	3.86
7.50	1.73	1.73	3.46	7.50	3.74	3.86
8.00	1.74	1.71	3.45	8.00	3.74	3.85

In Table 3 are shown values for *a*, *b*, *c*, and *d*. They were calculated from tables in *Methods of Analysis, A.O.A.C.*, 1935, *a* from the B. H. St. John table (rearranged) for immersion refractometer readings of alcohol solutions, *b* from the table calculated by J. A. Mathews for the refractometer readings of sucrose solutions, *c* from the Bureau of Standards alcohol-specific gravity table, and *d* from the Plato table.

The use of the values given for *a* and *c*, which are coefficients for pure alcohol, should lead to results that agree with the amounts of alcohol

TABLE 4.—*Direct determination of the refraction coefficient for the extract*

NO.	BREWERY	SPECIFIC GRAVITY FUNCTION OF DE-ALCOHOLIZED BEER (20°/20°C.), N'	DIFF. BETWEEN REFRACTOMETER READINGS OF DEALCOHOLIZED BEER AND WATER, 20°C. R'	REFRACTION COEFFICIENT FOR THE EXTRACT, b $\left(\frac{R'}{N'}\times 3.86\right)$
1	A	18.63	18.25	3.78
2	B	22.88	22.85	3.85
3	C	15.12	15.00	3.83
4	C	14.43	14.25	3.81
5	D	15.70	15.45	3.80
6	E	19.58	19.20	3.78
7	E	15.23	15.05	3.82
8	C	14.85	14.65	3.81
9	F	20.58	20.70	3.88
10	F	24.57	24.35	3.83
11	G	17.78	17.50	3.80
12	H	21.62	21.40	3.82
13	I	24.11	23.70	3.79
14	B	22.36	22.10	3.82
15	E	18.77	18.55	3.82
16	G	17.92	17.55	3.78
17	J	20.92	20.75	3.83
18	K	20.01	19.90	3.84
19	L	19.19	18.75	3.77
20	B	26.55	26.80	3.90

found by the standard method, if, as is apparently the case, the effects of the alcohol on specific gravity and refraction are the same in beer as in alcohol-water solutions, and if the amounts of other volatile substances in beer, such as acetic acid, are so small that their effects are negligible.

The extract in the beer must have the effect on the specific gravity represented by the values given for *d*, since the extract as calculated is only its effect on the specific gravity, divided by the coefficient *d*, inherent in the Plato table.

But the extract in the beer will probably not have the effect on the refractometer reading represented by the values given for *b*. Since the extract is defined from its effect on the specific gravity rather than on the refraction, the fact that actually it consists of different substances, such

as dextrines, proteins, salts, etc., will make its total effect on the refraction different from that of sucrose. It should be expected, moreover, that a different composition of the extract in different beers will result in different values for b, apart from any variation from the total amount of extract.

In Table 4 are shown results of direct determinations of the values of b for the twenty beer samples analyzed. A 100 cc. portion was dealcoholized by boiling down to about 30 cc., and made up to the original volume with distilled water. The specific gravity at 20/20°C. was determined with the pycnometer, and the difference in refractometer reading at 20°C. from that of water was found. With a constant value of 3.86 assumed for d, the value of b in each case was found by a proportion. The range found for b was from 3.77 to 3.90.

TABLE 5.—*Dependence of deviations of proposed formulas on refraction coefficient for extract*

(GRAMS PER 100 CC.)

NO.	REFRACTION COEFFICIENT OF EXTRACT b	BARTH		LEHMANN AND GERUM		BERGLUND ET. AL.	
		ALCOHOL DEVIATION	EXTRACT DEVIATION	ALCOHOL DEVIATION	EXTRACT DEVIATION	ALCOHOL DEVIATION	EXTRACT DEVIATION
19	3.77	+0.02	−0.07	−0.16	−0.10	−0.10	−0.12
12	3.82	+0.02	−0.03	−0.09	−0.08	−0.04	−0.09
18	3.84	+0.09	−0.01	−0.04	−0.05	+0.02	−0.06
9	3.88	+0.16	+0.01	+0.01	−0.04	+0.06	−0.06

As a demonstration of the fact that it is variations like these in the effect on the refraction of the extract of different beers that cause the deviations of the proposed formulas, there are shown in Table 5 the size and direction of these deviations in the case of four of the samples of about the same total extract, arranged in order of increasing values of b. It is clear from the regularity with which the deviations decrease if negative and increase if positive, with increasing b, that the reason for the inaccuracy of the proposed formulas is the assumption that b is the same in different beers.

NEW FORMULAS

For the usual range of alcohol and extract content, $a+c$ may be considered constant at 3.47, and d constant at 3.86. The denominators of the terms in equation 13 may then be taken as 3.47 modified by the fractions $b/3.86$ and $3.86/b$, applied in the first case to 1.80 parts of the quantity 3.47, and in the second case to 1.67 parts (these values are assumed for this calculation as average for c and a). On this basis a set of formulas containing the different values of b was calculated for the alcohol; they are given in Table 6.

TABLE 6.—*New formulas*

I. Alcohol (grams per 100 cc.)

When b = 3.72,	A = 0.2937 R −	0.2830 N
3.74	0.2929	0.2837
3.76	0.2921	0.2845
3.78	0.2913	0.2853
3.80	0.2905	0.2860
3.82	0.2898	0.2867
3.84	0.2890	0.2874
3.86	0.2882	0.2882
3.88	0.2874	0.2890
3.90	0.2866	0.2897
3.92	0.2859	0.2904
3.94	0.2851	0.2911

II. Extract (grams per 100 cc.)

When b = 3.72,	E = 0.1319 $(R+N)+K$
3.74	0.1316
3.76	0.1312
3.78	0.1309
3.80	0.1305
3.82	0.1302
3.84	0.1299
3.86	0.1295
3.88	0.1292
3.90	0.1289
3.92	0.1285
3.94	0.1282

A	K	A	K	A	K
1.0	+0.05	3.5	+0.08	6.0	+0.05
1.5	0.06	4.0	0.08	6.5	0.03
2.0	0.07	4.5	0.08	7.0	0.02
2.5	0.08	5.0	0.07	7.5	0
3.0	0.08	5.5	0.06	8.0	−0.02

For the extract formulas the following equation, obtained by the addition of equations 11 and 12, is more convenient than equation 14:

$$E = \frac{R+N}{b+d} - A\frac{a-c}{b+d}. \tag{15}$$

The formulas for extract in Table 6 are calculated from this equation. The second term, called K, varies with the alcohol, but does not vary sufficiently with b to make a difference of 0.01.

From these formulas it appears that a difference of 0.04 in b affects the alcohol to the extent of 0.09, and the extract to 0.03.

In Table 7 are shown the alcohol and extract, calculated by the new

formulas, for the twenty beer samples. The greatest deviation in the case of alcohol is 0.04, in the case of extract 0.02; the average deviations are 0.02 and 0.01, respectively. The deviations are both positive and negative in each case. Thus it appears that when the formulas used take account of the variable coefficient *b*, the method is capable of a high degree of accuracy.

TABLE 7.—*Results with new formulas*

(GRAMS PER 100 CC.)

NO.	ALCOHOL	ALCOHOL DEVIATION	EXTRACT	EXTRACT DEVIATION
1	3.64	+0.04	4.85	0
2	4.08	+0.03	5.97	−0.01
3	2.95	−0.03	3.92	−0.01
4	2.81	−0.01	3.71	+0.01
5	1.78	−0.04	2.31	+0.01
6	3.44	+0.02	5.10	+0.02
7	3.16	0	3.97	−0.01
8	2.78	−0.02	3.86	−0.01
9	3.60	+0.01	5.36	−0.01
10	3.38	0	6.41	−0.02
11	3.39	+0.01	4.61	−0.01
12	3.27	0	5.65	−0.01
13	3.84	+0.01	6.29	−0.02
14	3.23	+0.02	5.83	0
15	3.46	0	4.88	−0.01
16	3.36	+0.02	4.60	+0.01
17	3.09	+0.03	5.47	+0.01
18	3.22	+0.02	5.23	0
19	3.57	0	5.00	−0.01
20	4.83	−0.02	6.95	−0.02
Average deviation		0.02		0.01
Greatest deviation		0.04		0.02

EFFECT OF SEPARATE CONSTITUENTS OF EXTRACT

Several substances that might be considered constituents of beer extract were added to dealcoholized beer, and the effects on the refraction and specific gravity were measured. Solutions in water were made of lactic acid, of peptone (to represent protein), of maltose, of dextrin, obtained from beer by precipitation with alcohol, and of three salts, sodium chloride, potassium acid phosphate, and magnesium sulfate. The amount shown in the second column of Table 8 was contained in 50 cc. of the solution in each case. This volume of each was added to 100 cc. portions of the same dealcoholized beer and 50 cc. of distilled water was added to another 100 cc. portion of the dealcoholized beer. The specific gravities and refractometer readings were then determined.

The beer diluted with water had after dilution a specific gravity of 1.01724, and a difference in the refractometer reading from that of water of 17.10. The value for *b* was thus (17.10×3.86)÷17.24=3.83. By calculations from the effects of the various substances on the specific gravity and on the refractometer reading, values for *b* for the separate constituents were obtained; they are given in the last column of Table 8. Protein material, and to a lesser extent lactic acid, appear to have high values for *b;* that is, a relatively greater effect on refraction than on specific gravity. The salts have a smaller effect; maltose and dextrin have the same effect as sucrose.

TABLE 8.—*Effect of separate constituents of extract on refraction of beer*

SUBSTANCE	AMOUNT ADDED	CONCENTRATION	EFFECT ON SPECIFIC GRAVITY	EFFECT ON EXTRACT	EFFECT ON EXTRACT ÷CONCENTRATION	EFFECT ON REFRACTOMETER READING	EFFECT ON REFRACTOMETER READING ÷EFFECT ON EXTRACT
	grams	*g./100 cc.*		*g./100 cc.*			
Lactic Acid	0.30	0.20	0.00050	0.13	0.65	0.65	5.0
Peptone	0.30	0.20	0.00066	0.17	0.85	1.05	6.2
Maltose	2.16	1.44	0.00556	1.44	1.00	5.35	3.72
Dextrin	5.19	3.46	0.01328	3.44	0.99	12.80	3.72
Sodium Chloride	0.30	0.20	0.00144	0.37	1.8	1.00	2.7
Potassium Di-hydrogen Phosphate	0.30	0.20	0.00140	0.36	1.8	0.65	1.8
Magnesium Sulfate	0.30	0.20	0.00130	0.34	1.7	0.75	2.3
Average for three salts					1.8		2.3

It is clear from these results that a relatively greater proportion of protein or lactic acid in the extract will bring the average *b* for the whole extract a greater amount above 3.72, which is the value for four-fifths or more of the extract (maltose and dextrin), while a greater proportion of salts will make the increase above 3.72 somewhat less.

Twelve additional samples of beer, from eight breweries, were analyzed to compare values for *b* calculated from the composition of the extract with values determined directly, on dealcoholized portions. The extracts in these samples ranged from 3 to 8 grams per 100 cc. The direct determination of *b* was made as described for the first twenty samples. The beer itself, not dealcoholized, was analyzed for protein by the Kjeldahl method, for total acidity by titration, and for ash. Data are given in Table 9.

The assumptions were made that the effect on the refraction of the

total protein could be represented more or less closely by the value 6.2 found for peptone, that the effect of the total acidity would be that found for lactic acid, 5.0, that the effect of the mineral salts could be represented by the average value 2.3, applied to the percentage of ash found, and finally that the effect of the remainder of the extract, after protein, acid, and ash had been subtracted, would be that of maltose and dextrin, 3.72.

The method of calculation may be illustrated for Sample 21. The protein, acidity, and ash were multiplied by 0.85, 0.65, and 1.8, to obtain their effects on the extract according to the specific gravity. The fraction

TABLE 9.—*Comparison of directly determined refraction coefficient for extract with value calculated from extract, protein, acidity, and ash*

NO.	EXTRACT	PROTEIN	TOTAL ACIDITY AS LACTIC ACID	ASH	CALCULATED COEFFICIENT	DETERMINED COEFFICIENT
	g./100 cc.	*g./100 cc.*	*g./100 cc.*	*g./100 cc.*		
21	6.86	0.73	0.23	0.27	3.87	3.90
22	6.89	0.75	0.23	0.28	3.87	3.90
23	6.18	0 62	0.21	0.18	3.89	3.88
24	6.16	0.56	0.19	0.18	3.86	3.88
25	4.54	0.35	0.18	0.12	3.85	3.82
26	5.60	0.42	0.15	0.23	3.78	3.81
27	5.56	0.60	0.17	0.26	3.85	3.84
28	2.97	0.28	0.10	0.16	3.80	3.77
29	5.67	0.70	0.19	0.25	3.89	3.88
30	8 09	0.56	0.18	0.26	3.80	3.80
31	5.20	0.41	0.15	0.20	3.81	3.79
32	5.72	0.49	0.17	0.23	3.82	3.81

of the total extract which each of these constituted was found by dividing each result by the extract, 6.86. The sum of these fractions, 0.0904 0.0700, and 0.0219, was subtracted from 1, giving 0.8177 as the fraction of maltose and dextrins; each of the four fractions was then multiplied by its value for *b*, and the results added. The calculated value thus obtained was 3.87, comparable with the directly determined value 3.90.

Fairly good agreement was obtained for the twelve samples, considering the many assumptions. The greatest difference of the value calculated from the analysis from that determined in the dealcoholized beer was 0.03; the average difference was 0.02.

It should be noted that it is not the percentage in the beer of protein, etc., which is used to study its effect on the refraction coefficient, but the fraction which it constitutes of the total extract, figured from its effect on the specific gravity. Also a higher fraction of protein, for example, in one beer than in another, will make the value of *b* high only if the other constituents are the same; its effect may be balanced by that of a higher fraction of ash, as in Sample 27 compared with Sample 25.

DISCUSSION

The precision of the method is not equal to that of the standard distillation method, even when the correct value for the refraction coefficient of the extract is known. Still for all but the most exact work an accuracy better than 0.05 per cent is satisfactory. And when the method is used as a check, it has the great value of indicating any loss that may occur in the process of distillation, since it is a determination of the alcohol while it is still in the beer, from its effect in the presence of other substances.

The extract calculation shows about twice the accuracy of the alcohol calculation. The reason for this is the fact that a certain specific gravity change, for example 0.00004, corresponds to the effect of about twice as much alcohol as extract: 0.01 extract, and a little over 0.02 alcohol. There would consequently be no gain in accuracy if the result obtained for extract were used with the specific gravity of the beer to calculate the alcohol. This ratio of effects of alcohol and extract on the specific gravity is the source of the numbers 2 and 0.9 in the Lehmann and Gerum formulas.

Formulas can be obtained for use in rapid approximate analyses, by taking an average value for b. A large number of analyses, of samples from 17 breweries, gave an average of 3.83 for b. For this value the formulas are:

$$A = 0.2894R - 0.287N. \tag{16}$$

$$E = 0.130(R + N) + K. \tag{17}$$

The accuracy of these formulas will be similar to that of the proposed formulas discussed in the first part of this paper. The maximum errors due to the use of an average coefficient, apart from experimental error, that is, can be estimated, from the range of values found for b, as about ± 0.18 alcohol, and ± 0.06 extract. These are comparable with the range of deviations of the other formulas, shown in Table 2.

Under special circumstances another value for b than the average of 3.83 could be assumed. In control analyses, of a number of samples of the same type of beer, the value for that beer, once determined, could be used for accurate calculations of all the samples. Table 10 shows results of tests made to confirm the constancy of b for the same type of beer. The separate samples were tested at different times. The difference in refraction coefficient of the extract between the two types of beer is no doubt due to differences in materials or mashing processes, which result in a different composition of the extract.

If b is to be determined directly for each sample, the residue from the alcohol distillation can be used. It need be made up only approximately to volume, since it is the ratio between refraction and specific gravity which is required. The assumption is involved that no alcohol remains in

the residue. If alcohol should remain, the result for alcohol by the standard method would of course be too low, and the result calculated from the refractometer formula would be too low also, because the presence of alcohol in the dealcoholized beer would make the value found for b too high, and when a high value of b is used the calculated alcohol is low.

If a complete analysis of a beer sample is made, including protein, acidity, and ash, the value of b to be used can be calculated as shown, but it will be less accurate than a directly determined value, probably because of the different effects, at present unknown, of different kinds of proteins, and other substances, on the refraction.

TABLE 10.—*Refraction coefficient of extract for two different types of beer from the same brewery*

BREWERY	TYPE OF BEER	SPECIFIC GRAVITY FUNCTION OF DEALCOHOLIZED BEER (20°/20°C.) N'	DIFF. BETWEEN REFRACTOMETER READINGS OF DEALCOHOLIZED BEER AND WATER, 20°C. R'	REFRACTION COEFFICIENT FOR THE EXTRACT, b $\left(\frac{R'}{N'}\times 3.86\right)$
B	1	21.75	21.85	3.88
B	1	21.92	22.15	3.90
B	1	21.73	21.85	3.88
B	1	21.65	21.80	3.89
B	1	22.12	22.25	3.88
			Av. for 1:	3.89
B	2	22.36	22.10	3.82
B	2	21.59	21.35	3.82
B	2	22.46	22.05	3.79
B	2	22.34	21.90	3.78
			Av. for 2:	3.80

Ackermann's method for alcohol and extract, in terms of the refraction of the beer and distillate (equation 10), is analogous to the standard method, in which the specific gravity of the beer and distillate are used. There are two main objections to his method. The precision of refractometer readings is less than that of specific gravity determination, especially on the distillate, where 0.1 in the scale reading is equivalent to about 0.06 alcohol, while 1 mg. in the weight of a 50 cc. pycnometer filled with distillate corresponds to a little over 0.01 alcohol. A constant effect of the extract on the refraction, with $b=3.89$, is assumed in Ackermann's formula; this is incorrect so long as the extract is expressed in Balling or Plato degrees, based on the specific gravity.

SUMMARY

The alcohol and extract in beer can be calculated from the refraction and specific gravity. The effect of the extract of different beers on the

refraction is not the same, but depends on the proportions of proteins, acids, and salts in the extract. If this effect is determined for each sample, from the refraction and specific gravity of a dealcoholized portion, the calculated alcohol will agree with the result by the standard method to within 0.04 grams per 100 cc., and the extract to within 0.02. The effect of the extract on the refraction can also be calculated from the percentages of protein, acidity, and ash, when these have been determined. For the same type of beer from the same brewery the effect is constant.

THE INSOLUBLE RESIDUE IN THE POTASSIUM CHLOROPLATINATE OBTAINED IN THE ANALYSIS OF CERTAIN FERTILIZERS FOR POTASH*

By H. R. ALLEN (Agricultural Experiment Station, Lexington, Ky.)

In a previous paper[1] results from the use of silica and platinum dishes in the method for the determination of potash in fertilizers were presented. Ignition was conducted at the lowest practical temperature to assure a perfectly white residue. In some cases, particularly when organic material was present in the fertilizer, considerable insoluble residue resulted, the amount of which was determined by the difference in weight of the crucible originally and after washing out the K_2PtCl_6. Since the official method does not specify this latter procedure, any insoluble material would be counted as K_2PtCl_6.

The present paper is an investigation of means to eliminate or reduce the insoluble residue by ignition at higher temperatures either over the Meker burner or in the electric muffle or by a double ignition with sulfuric acid. The official method for the determination of potash in fertilizers[2] was used, except in the ignition procedure and in washing out the K_2PtCl_6. Most of the samples used for comparative study were complete fertilizers that had shown insoluble material on a previous determination.

After evaporation on the water bath of a 25 ml. aliquot, corresponding to a 0.25 gram sample, the dishes were placed on a hot plate and heated slowly until frothing began, when they were transferred to burners equipped with the Purdue burner top[3] and the heat was gradually increased until all organic material was removed from the sides of the dishes. Ignition was continued over Tirrill burners until the residue was white. The procedure so far was the usual low temperature ignition. Ignition was continued at higher temperatures either over the Meker type

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.
[1] *This Journal*, 20, 101 (1937).
[2] *Methods of Analysis*, A.O.A C., 1935, 30.
[3] *Ind. Eng. Chem. Anal. Ed.*, 7, 119 (1935).

(Fisher) burner or in the electric muffle. Dishes were placed on a nichrome triangle, which rested on a chimney guard (E. & A. catalog No. 19401) which enclosed the Fisher burner. The bottom of the dish was about $\frac{3}{4}$ inch above the top of the burner, and the full heat of the burner was applied for 1–2 minutes. Ignition in the electric muffle was for a period of 15 minutes at a full red heat (750° C. as determined by a seger cone). The double ignition with sulfuric acid was conducted as follows: After ignition over the Tirrill burner the dishes were cooled, enough sulfuric acid (1+1) to wet the residue (usually 6–8 drops) was added, and ignition was conducted carefully over low flame until all SO_3 fumes were driven off. Ignition was finished by one of the procedures described above. In this procedure it is thought that the temperature attained over the Tirrill burner is the lowest; that over the Fisher burner, next; and that in the muffle, the highest. Of course, these temperatures were not controlled with any degree of precision.

Ignition in a platinum dish over a Fisher burner or in the muffle usually fused the residue, and in some instances it cooled to a hard mass. A small amount of hot water was added, and the residue was loosened from the dish and broken up by means of a glass rod having a flattened end. The rod was washed with a fine stream of water, and hot water and a few drops of hydrochloric acid were added. The mixture was allowed to stand about 30 minutes before the platinum solution was added, and the dish was then placed on the bath. In the case of the silica dishes, a rubber policeman was used in place of the glass rod. The K_2PtCl_6 was allowed to stand for at least 15 minutes in the acid alcohol before filtering. After

TABLE 1.—(A) *K_2PtCl_6 and insoluble residue obtained by washing out with hot water*—sulfates ignited in different ways*
(Results expressed as mg.)

SAMPLE NUMBER	SILICA DISH						PLATINUM DISH			
	TIRRILL BURNER USUAL		TIRRILL BURNER DOUBLE IGNITION		MUFFLE 750° C. DOUBLE IGNITION		MUFFLE 750° C. USUAL		MUFFLE 750° C. DOUBLE IGNITION	
	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE
1	99.9	6.8	103.5	4.6	—	—	104.7	1.6	—	—
2	80.4	6 2	81.1	5.1	82.8	3.7	84.0	1.0	84.1	0.0
3	35.9	1.9	36.8	5.3	—	—	37.4	4.6	37.3	4.8
4	232.3	3.0	233.4	1.7	—	—	232.4	0.3	—	—
5	66.8	8.5	68.8	8.8	68.8	2.8	68.8	4.9	68.5	0.9
6	120.4	4.5	120.2	4.4	—	—	121.9	0.0	121.9	0.2
7	49.2	8.0	51.0	7.7	47.5	9.4	50.4	3.6	51.0	2.6
8	81.1	6.7	84.9	4.6	—	—	84.5	0.5	—	—
9	48.2	6.5	50.6	2.8	—	—	50.4	2.4	—	—

* All findings from any one sample are from the same solution.

the K_2PtCl_6 had been weighed the soluble matter was leached out with boiling water, but each addition of water was allowed to run out before more was added. After solution of all the K_2PtCl_6 was apparent, water was added about four times to ensure complete solution. After the filter crucible had been dried and weighed, tests were made in a number of instances to determine whether all the water-soluble material was dissolved. The filter pads were re-washed about six times with boiling water, and the crucibles were dried and weighed. Loss in weight averaged less than 0.2 mg.

TABLE 1.—(B) *K_2PtCl_6 and insoluble residue obtained by washing out with hot water—sulfates ignited in different ways*
(Results expressed as mg.)

SAMPLE NUMBER	SILICA DISH				PLATINUM DISH					
	TIRRILL BURNER USUAL		FISHER BURNER DOUBLE IGNITION		TIRRILL BURNER USUAL		FISHER BURNER USUAL		MUFFLE 750° C.	
	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE
10	51.9	9.0	51.2*	3.0	51.5*	4.5	—	—	51.1*	0.7
11	38.3	5.0	—	—	—	—	—	—	39.5	0.0
12	59.2	5.2	60.6*	3.5	—	—	—	—	60.7*	0.5
13	49.7	8.2	50.2*	4.4	46.5*	7.3	—	—	49.5*	0.0
14	75.1	5.4	76.1*	2.1	74.4*	4.5	—	—	76.4*	0.3
15	68.2	5.6	70.8*	5.4	71.9*	3.5	73.0*	2.6	73.3*	4.4
16	66.3	5.9	69.1*	5.2	69.8*	3.5	70.9*	1.5	69.9*	2.6
17	69.1	7.2	73.5	4.6	71.3*	7.8	72.9	7.6	74.5*	0.0
18	68.2	7.1	72.3*	3.2	72.1	4.4	74.2	0.0	73.6*	0.8
19	—	—	36.9*	3.7	36.7	6.0	36.9	0.0	37.2*	1.7
20	46.8	6.6	48.5	3.1	—	—	—	—	48.6	0.9
21	—	—	—	—	63.1*	4.4	64.0*	3.8	63.7*	3.7
22	—	—	—	—	78.2*	0.0	79.0*	0.0	78.1*	0.0

* From the same solution for each sample.

Tables 1.—(A), (B), and (C)—give the results of various modes of ignition.

The potash content of a mixture containing K_2SO_4, superphosphate, finely ground tobacco leaf, and tankage was determined. One gram of superphosphate, 0.5 gram of tobacco, and 0.25 gram of tankage were put into a 250 ml. flask for digestion, and the potash content was determined. To the same amounts of these materials in another flask, 0.6250 gram of a pure potassium sulfate, dried at 120° C., was added, and the potash content was determined. Results are given in Table 2. A determination of the potassium sulfate alone gave 174.1 mg. of K_2PtCl_6 from a 25 ml. aliquot; theory, 174.4 mg.

The findings show a decided tendency for the insoluble residue to be

smaller when the temperature of ignition is higher. For instance, the average weight from 19 samples, ignited as usual over the Tirrill burner, was 6.1 mg. against 4.3 by double ignition over the Tirrill or Fisher burner. In only two instances out of the 19 was the weight from ignition over the Tirrill burner smaller than the other.

In the same 19 analyses, the average weights of K_2PtCl_6 were 73.9 and 75.9 mg., respectively. Adding the weights of insoluble residue gives 80.0 and 82.0 mg., respectively. So it appears that the extra ignition did not cause loss of potassium. Indeed, the amount of potassium returned from

TABLE 1.—(C) *K_2PtCl_6 and insoluble residue obtained by washing out with hot water—sulfates ignited in different ways*
(Results expressed as mg.)

SAMPLE NUMBER	SILICA DISH				PLATINUM DISH	
	TIRRILL BURNER, DOUBLE IGNITION		MUFFLE 750° C.*		MUFFLE 750° C.	
	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE
23	—	—	91.7	8.7	94.1	2.3
24	91.3	0.8	91.0	2.4	—	—
25	—	—	58.3	7.8	59.4	4.8
26	58.3	7.3	61.0	1.8	—	—
27	—	—	63.4	7.8	67.2	1.9
28	43 5	0.5	43.4	0.5	—	—
29	—	—	49.8	7.8	53.1	2.5
30	61.9	0.5	63.8	4.9	—	—
31	73.5	3.6	76.0	1.7	—	—
32	41.6	0.7	41.5	2.3	—	—
33	—	—	71.1	8.1	72.0	0.0

* 1.5 ml. of normal NaOH used.

the extra ignition seems to be larger; of the 21 analyses, only 3 yielded less K_2PtCl_6 by extra ignition.

The average weight of K_2PtCl_6 obtained from 19 samples in silica dishes by double ignition over the Tirrill or Fisher burner was 75.9 mg., and the weight of the same samples in platinum, ignited in the muffle, was 76.3 mg. The weights of the insoluble residue were 4.3 and 1.6 mg., respectively. The average weight of K_2PtCl_6 obtained from 5 samples in silica dishes over the Fisher burner was 64.5 mg. against 65.6 mg. in platinum dishes. The weights of the insoluble residues were 4.4 and 2.3 mg., respectively. The average weight of K_2PtCl_6 obtained from 5 samples in silica dishes in the muffle was 66.9 mg. against 69.2 mg. in platinum dishes. The weights of the insoluble residue were 8.0 and 2.3 mg., respectively.

A partial record was kept of the amount of insoluble material found in routine potash determinations made in a set of silica dishes. The results

TABLE 2.—*K_2PtCl_6 and insoluble residue obtained in the analysis of a mixture of superphosphate, tobacco powder, and tankage, with and without the addition of K_2SO_4*
(Results expressed in mg.)

	Silica dish				Platinum dish							
	Tirrill burner, double ignition		Fisher burner, double ignition		Tirrill burner, usual		Fisher burner, usual		Muffle 750° C. usual		Muffle 750° C. double ignition	
	K_2PtCl_6	Insoluble residue	K_2PtCl_6	Insoluble residue	K_2PtCl_6	Insoluble residue	K_2PtCl_6	Insoluble residue	K_2PtCl_6	Insoluble residue	K_2PtCl_6	Insoluble residue
Superphosphate, tobacco, and tankage	(8.6)	—	(8.6)	—	8.2	1.2	8.8	0.8	8.7	0.2	8.5	0.2
Superphosphate, tobacco, tankage, and K_2SO_4	178.8	4.5	179.0	4.1	180.3	2.8	181.9	1.3	182.1	0.2	182.5	0.4
					181.1	1.3	181.5	0.0	182.0	0.0	182.4	0.0
Theory	183.0*		183.0		182.6		183.2		183.1		182.9	

* Assuming that 8.6 mg. of K_2PtCl_6 would come from K in the superphosphate, tobacco, and tankage.

are shown in Table 3. The quantity of insoluble residue seems to be larger when the silica dishes have been in use for some time.

In the analyses of the laboratory mixture, Table 2, recovery of potassium was nearest theoretical when the insoluble residue was counted as K_2PtCl_6, and if any loss occurred by strong ignition it was trifling.

TABLE 3.—*Frequency of occurrence of different weights of insoluble matter—ignition in silica dishes*

	DISHES USED 25 TIMES		SAME DISHES AFTER LONGER USE	
WEIGHT OF INSOLUBLE MATTER	NUMBER OF ANALYSES	PERCENTAGE OF TOTAL	NUMBER OF ANALYSES	PERCENTAGE OF TOTAL
mg.				
0 –1	113	60.7	65	38.0
1.1–2	17	9.1	32	18.7
2.1–4	18	9.7	37	21.7
4.1–6	11	6.0	25	14.6
Over 6	27	14.5	12	7.0
Total	186	100.0	171	100.0

In one series of duplicate analyses the K_2PtCl_6 was left in contact with the acid alcohol for 15 minutes and overnight in a desiccator in another series. Platinum dishes and the Tirrill burner ignition were used. The results are given in Table 4.

TABLE 4.—*K_2PtCl_6 and insoluble residue obtained when in contact with acid alcohol for different periods of time*

SAMPLE NUMBER	15 MINUTES		OVERNIGHT	
	K_2PtCl_6	INSOLUBLE RESIDUE	K_2PtCl_6	INSOLUBLE RESIDUE
	mg.	*mg.*	*mg.*	*mg.*
34	47.0	5.2	46.4	5.7
35	50.0	8.1	49.9	6.1
36	65.8	5.4	66.5	5.0
37	97.6	3.3	98.4	2.7

The quantities of K_2PtCl_6 from six determinations made in platinum dishes with the Tirrill burner ignition were placed on the same ashless filter paper and washed as usual, and the K_2PtCl_6 was leached out and the residue examined. From 21.0 mg. of residue, 3.0 mg. was not dissolved in hydrochloric acid and 2.1 mg. of this was silica. In the filtrate 1.6 mg. of Fe_2O_3 and 5.5 mg. of P_2O_5 were found. Sodium was present. Calcium, magnesium, and potassium were not found.

SUMMARY

In a majority of the analyses the following relationships appeared:

The quantity of insoluble residue was smaller with higher temperature of ignition except when silica dishes were used in the muffle.

The quantity of K_2PtCl_6 was larger with higher temperature of ignition.

More potassium, with less insoluble residue, was obtained in platinum than in silica dishes, with higher temperature of ignition.

It appears that in the determination of potassium in fertilizers ignition of the sulfates at about 750° C. is preferable to ignition at a lower temperature.

EFFECT OF VARIOUS CARBOHYDRATE MATERIALS ON THE DETERMINATION OF LIGNIN BY THE FUMING HYDROCHLORIC ACID METHOD*

By Max Phillips and M. J. Goss

The methods most commonly used for the determination of lignin are based on the assumption that when a lignified plant material is treated with fuming hydrochloric acid (42–43% HCl) or strong sulfuric acid (72% H_2SO_4 is generally employed) all the carbohydrates and other substances associated with the lignin are hydrolyzed into water-soluble products, and the lignin is left as an insoluble residue. That this is not entirely true has been known for many years, and several investigators have called attention to the shortcomings of these methods. It is known, for example, that certain substances removable from a plant material by extraction with a 1:2 alcohol-benzene solution, as well as certain nitrogenous complexes when treated with either fuming hydrochloric acid or 72 per cent sulfuric acid, afford products as resistant to hydrolysis as lignin itself.

It was first pointed out by Paloheimo[1] that certain carbohydrates such as fructose and sucrose, when subjected to the action of fuming hydrochloric acid, yield insoluble humus-like substances. According to this investigator, fructose yields 25 per cent of its weight of an insoluble material.

Norman and Jenkins[2] show that xylose, arabinose, or substances yielding these sugars on hydrolysis, as well as fructose and sucrose when treated with 72 per cent sulfuric acid for 16 hours at 20° C. or less and then with boiling 3 per cent sulfuric acid afford some insoluble materials as resistant as lignin. The disturbance produced by xylose was found to be considerably greater than that caused by sucrose.

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1 *Biochem. Z.*, 214, 161 (1929).

2 *Nature*, 131, 729 (1933); *Biochem. J.*, 28, 2147 (1934).

Hilpert and Littman,[1] without mentioning the earlier findings of Paloheimo, reported on the action of concentrated mineral acids on sugars in relation to the determination of lignin. When various carbohydrate materials were treated with 72 per cent sulfuric acid for 48 hours at 20–22° C., the following quantities (expressed as per cent of sample taken) of insoluble humin-like products were obtained: Arabinose 9.22, xylose 36.30–36.88, xylan 33.44, glucose 0.81, mannose 0.96, galactose 0.09, fructose 25.03, sucrose 13.10–14.21, starch 0.94, and inulin 24.86. When treated with fuming hydrochloric acid at 0° C. for 48 hours xylose afforded 21.58 per cent of an insoluble material, fructose yielded 15.98 per cent, sucrose 10.90 per cent, and starch 0.46 per cent. In a later publication Hilpert and Littman[2] showed that the action of 72 per cent sulfuric acid on carbohydrates is greatly affected by temperature so that at 6° C. fructose and xylose yield only 0.26 per cent and 0.01 per cent respectively of insoluble humin-like residues.

Schmidt-Nielsen and Opperud[3] found that fucose and cellobiose afforded no insoluble residue when treated with 72 per cent sulfuric acid.

Bamford and Campbell[4] found that xylose, fructose and sucrose when treated with 72 per cent sulfuric at 10° C. for 5 hours yielded only negligible quantities of insoluble residues.

In a paper published by Goss and Phillips[5] a method for the quantitative estimation of lignin is described. This method, which has been adopted by the A.O.A.C. as a tentative method, makes use of fuming hydrochloric acid for the hydrolysis of the cellulose and the associated carbohydrates under definite conditions as to temperature and time of reaction. In view of the fact that it has been reported by several investigators that strong mineral acids, such as are employed in the determination of lignin, react with certain carbohydrates with the formation of insoluble humin-like materials, it seemed of interest to determine whether under the conditions prescribed by the method of Goss and Phillips such insoluble products are obtained. Accordingly, the experiments described in this paper were carried out.

EXPERIMENTAL

In all the experiments recorded in this paper, the procedure recommended by Goss and Phillips for the quantitative estimation of lignin was followed except, of course, the preliminary successive extractions of the sample with a 1:2 alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution, and the subsequent determinations of the percentages ash and nitrogen in the insoluble residual material were omitted. Briefly, the experimental procedure is as follows: The weighed

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[1] *Ber.*, 67, 1551 (1934).
[2] *Ibid.*, 68, 16 (1935).
[3] *Kgl. Norske Videnskab. Selskb. Forh*, 9, No. 5, 16–19 (1935) through *C. A.*, 31, 2419 (1936).
[4] *Biochem. J.*, 30, 419 (1936).
[5] *This Journal*, 19, 341 (1936).

sample was placed in one of the tubes of the apparatus of Goss and Phillips (cooled with ice), 50 cc. of fuming hydrochloric acid (d. 1.212–1.223 at 15° C.) was added, and the mixture was agitated by means of a current of dry HCl gas for 2 hours. The reaction mixture was allowed to remain for 24 hours at a temperature of +8° C. to +10° C. It was then

TABLE 1.—*Effect of successive treatments of various carbohydrate materials with 42–3% HCl in the cold and with boiling 5% HCl*

SUBSTANCE	WT. OF CARBOHYDRATE		COLOR OF SOLUTION AFTER 24 HOURS STANDING WITH 42–3% HCl		PRECIPITATE OBTAINED			
					SERIES NO. 1		SERIES NO. 2	
	SERIES NO. 1	SERIES NO. 2	SERIES NO. 1	SERIES NO. 2		WT. OF SUBSTANCE		WT. OF SUBSTANCE
	gram	*gram*			*gram*	*per cent*	*gram*	*per cent*
Arabinose	0.2000	0.5000	Faint Yellow	Yellow	0	0	0	0
Xylose	0.2000	0.5000	Light Yellow	Light Brown	0	0	0	0
Glucose	0.2000	0.5000	Color-less	Color-less	0	0	0	0
Mannose	0.2000	0.5000	Faint Yellow	Yellow	0	0	0	0
Galactose	0.2000	0.5000	Color-less	Light Yellow	0	0	0	0
Fructose	0.2000	0.5000	Brown	Dark Brown	0.0018	0.90	0.0246	4.92
Sucrose	0.2000	0.5000	Light Brown	Brown	0.0006	0.30	0.0078	1.56
Maltose	0.2000	0.5000	Faint Yellow	Light Yellow	0	0	0	0
Starch	0.2000	0.5000	Faint Yellow	Light Yellow	0	0	0	0
Inulin	0.2000	0.5000	Brown	Dark Brown	0.0025	1.25	0.0209	4.18
Pectin	0.2000	0.5000	Light Brown	Brown	0	0	0.0046	0.92
Cellulose (Filter Paper)	0.2000	1.0000	Color-less	Color-less	0	0	0.0020	0.20

diluted with water to a volume of 500 cc. and boiled under a reflux condenser for 1 hour. The solution was allowed to cool to room temperature, filtered on a weighed Gooch crucible, dried at 105° C., and reweighed.

Two series of experiments were conducted. In the first series 0.2 gram of carbohydrate material was used and in the second series 0.5 gram. The results obtained are recorded in Table 1.

It will be observed (Table 1) that in the first series of experiments, the only carbohydrates yielding insoluble precipitates were sucrose, fructose, and inulin. The amount afforded by sucrose, however, was small and practically negligible. In the second series of experiments where 0.5 gram samples of carbohydrate materials were used, insoluble precipitates were again obtained in the case of sucrose, fructose, and inulin. In this series, pectin also yielded a small quantity of insoluble material. It was observed in connection with the experiment with pectin that this substance had the tendency to agglutinate and it was necessary to break up the particles mechanically in order to obtain the full effect of the hydrolytic action of the fuming hydrochloric acid. The precipitate afforded by cellulose was small and well within the experimental error.

In the second series of experiments, where the concentration of the carbohydrate materials with respect to the fuming hydrochloric acid was two and one-half times as great as in the first series, the percentages of insoluble materials obtained from fructose, sucrose, and inulin were considerably greater. It may be pointed out, however, that the percentages of insoluble products obtained from fructose or from those carbohydrates yielding fructose on hydrolysis were considerably less than those recorded in the literature.

In order to ascertain whether the various carbohydrate materials listed in Table 1 might behave differently in the presence of lignified cellulosic material, two series of experiments were carried out exactly as previously described, except that in addition to the carbohydrate material under investigation a known weight of wheat straw was used. This wheat straw had previously been extracted successively with a 1:2 alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution; ground to pass an 80-mesh sieve, and dried at 105° C. In every experiment the 0.6 gram of straw used contained 0.1370 gram of lignin (determined by the method of Goss and Phillips). In the first series of experiments 0.2 gram of carbohydrate material and 0.6 gram of wheat straw were used, and in the second, 0.5 gram of the carbohydrate plus the same amount of wheat straw. The results obtained are recorded in Table 2.

It will be observed (Table 2) that the results obtained in the two series of experiments are, in the main, in agreement with those recorded in Table 1. In the first series there was an apparent increase in the lignin content of the wheat straw only in the case of fructose or in those carbohydrates yielding fructose on hydrolysis. The increases expressed as per

cent of weight of the sample were as follows: Fructose 1.88, sucrose 0.88, inulin 1.82. In the second series of experiments, increases in the apparent lignin content of the wheat straw were also obtained in the experiments with fructose, sucrose, and inulin. In the experiment where pectin had been added to the wheat straw, the apparent increase in the lignin content of the straw was negligible. The increases expressed as per cent of

TABLE 2.—*Effect on yield of lignin from wheat straw of successive treatments of various carbohydrate materials with 42–3% HCl in the cold and with boiling 5% HCl*
(In every experiment in addition to the carbohydrate, 0.6 gram of wheat straw was added which contained *0.1370* gram lignin)

CARBOHYDRATE ADDED TO WHEAT STRAW	WT. OF CARBOHYDRATE ADDED		YIELD OF LIGNIN		INCREASE IN WT. OF LIGNIN			
					SERIES NO. 1		SERIES NO. 2	
	SERIES NO. 1	SERIES NO. 2	SERIES NO. 1	SERIES NO. 2		WT. OF SAMPLE		WT. OF SAMPLE
	gram	*gram*	*gram*	*gram*	*gram*	*per cent*	*gram*	*per cent*
Arabinose	0.2000	0.5000	0.1365	0.1361	0	0	0	0
Xylose	0.2000	0.5000	0.1365	0.1375	0	0	0.0005	0.04
Glucose	0.2000	0.5000	0.1358	0.1366	0	0	0	0
Mannose	0.2000	0.5000	0.1350	0.1369	0	0	0	0
Galactose	0.2000	0.5000	0.1367	0.1353	0	0	0	0
Fructose	0.2000	0.5000	0.1521	0.1748	0.0151	1.88	0.0378	3.43
Sucrose	0.2000	0.5000	0.1441	0.1587	0.0071	0.88	0.0217	1.97
Maltose	0.2000	0.5000	0.1361	0.1357	0	0	0	0
Starch	0.2000	0.5000	0.1361	0.1359	0	0	0	0
Inulin	0.2000	0.5000	0.1516	0.1687	0.0146	1.82	0.0317	2.88
Pectin	0.2000	0.5000	0.1357	0.1405	0	0	0.0035	0.31

weight of the sample obtained in the second series of experiments were as follows: Fructose 3.43, sucrose 1.97, inulin 2.88, and pectin 0.31.

From the results obtained in this investigation, it appears that when arabinose, xylose, glucose, mannose, galactose, maltose, starch, and cellulose (alone or in the presence of lignified cellulosic material), are treated with fuming and dilute hydrochloric acid in accordance with the procedure of Goss and Phillips for the quantitative estimation of lignin, they do not afford insoluble humin-like precipitates and therefore would not interfere with the determination of lignin in plant materials containing these carbohydrates. The fact that neither arabinose nor xylose yields

any insoluble products under the experimental conditions recommended by Goss and Phillips for the determination of lignin is of special interest as these two carbohydrates, as well as cellulose, are always associated (as arabans and xylans) with lignin in plants. The only carbohydrate materials that might cause any interference with the determination of lignin are fructose, sucrose, and inulin. The disturbance produced by pectin is small and practically negligible. From the results of the second series of experiments recorded in Table 2 where the carbohydrate added to the wheat straw amounted approximately to 45 per cent of the weight of the sample, the percentages increase in the lignin content of the samples were as follows: Fructose 3.43, sucrose 1.97, and inulin 2.88. It is, however, hardly necessary to point out that no lignified plant material ever naturally contains any such large percentages of either sucrose, fructose, fructosans or fructosides. Moreover, the method of Goss and Phillips for the quantitative estimation of lignin calls for a preliminary successive extraction of the plant material with 1:2 alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution. This treatment, it may reasonably be assumed, would remove quite effectively any sucrose, fructose, fructosans, or fructosides that might be present. It may, therefore, be concluded as a result of this investigation that in the determination of lignin by the method of Goss and Phillips, the carbohydrates generally associated with lignin in the plant do not interfere appreciably with the determination.

SUMMARY

A study was made of the action of 42–43 per cent and 5 per cent hydrochloric acid on various carbohydrates in relation to the determination of lignin by the method of Goss and Phillips. Insoluble humin-like materials were obtained only in the case of fructose, sucrose, inulin, and pectin, the percentages of insoluble material being, however, much less than those recorded in the literature. It is pointed out that the preliminary extraction of the plant material with hot water and 1 per cent hydrochloric acid would remove effectively any of the interfering carbohydrates and would, therefore, not interfere appreciably with the determination.

The writers wish to express their thanks to Dr. E. Yanovsky of the Carbohydrate Research Division of this Bureau for supplying the carbohydrates used in this investigation.

THE HYDROLYSIS OF WILLSTÄTTER LIGNIN FROM WHEAT STRAW*

By MAX PHILLIPS

It is generally accepted that lignin, or at least the lignin nucleus, is very resistant to the hydrolytic action of strong mineral acids, and several

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methods for its isolation and quantitative estimation are based on this fact. Although the literature on the chemistry of lignin is now extensive, no paper has appeared dealing with the hydrolysis of the lignin complex as a whole, although there is some information available on the removal by hydrolysis, of some of the constituent groups. Heuser[1] and co-workers succeeded in demethoxylating lignin by heating it with 5 per cent hydrochloric acid in a sealed tube at a temperature of 170–180° C. Hägglund and Björkman[2] and later Hägglund and Rosenqvist[3] distilled Willstätter lignin with 12 per cent hydrochloric acid and obtained a distillate that gave a precipitate with phloroglucinol, barbituric acid, and thiobarbituric acid. The substance present in the distillate was later identified by Freudenberg and Harder[4] as formaldehyde, which, according to these investigators, resulted from the hydrolysis of a methylene dioxide group supposedly present in the lignin molecule.

In connection with another investigation now in progress in this laboratory, it was observed that if lignin (isolated from wheat straw by the fuming hydrochloric acid method) were subjected to an alternate treatment with cold fuming hydrochloric acid and boiling 5 per cent hydrochloric acid, and the general procedure recommended by Goss and Phillips[5] for the quantitative estimation of lignin followed, a considerable loss in the weight of the lignin resulted. By repeating this alternate treatment four successive times and using in each case the residual lignin from the preceding experiment, losses in weight were again obtained, although they were of a lesser magnitude than those found in the first experiment. The percentages methoxyl in the residual lignin in all the experiments remained practically the same as in the original lignin, thus indicating that the loss in weight was not due merely to the removal of some impurity associated with the lignin, but rather that a degradation of the lignin complex as a whole had taken place. The results obtained, although of a somewhat preliminary nature, are nevertheless believed to be of sufficient importance to warrant presenting them at this time.

EXPERIMENTAL

Wheat straw was ground to pass an 80-mesh sieve and extracted successively with a 1:2 alcohol-benzene solution, hot water, and one per cent hydrochloric acid solution, according to the procedure described in a previous communication.[6] Twenty grams of the extracted straw was then treated in the cold with one liter of fuming hydrochloric acid (d. 1.212–1.223 at 15° C.) and allowed to remain at a temperature of +8 to +10° C. for 24 hours. The reaction mixture was then diluted with a sufficient quantity of water required to make a 5 per cent hydrochloric

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[1] *Cellulosechem*, 1, 49 (1920); *Ibid.*, 2, 81 (1921).
[2] *Biochem. Z*, 147, 74 (1924).
[3] *Ibid.*, 179, 376 (1926).
[4] *Ber.*, 60, 581 (1927).
[5] *This Journal*, 19, 341 (1936).
[6] *Ibid.*, 18, 386 (1935).

acid solution and boiled under the reflux condenser for one hour. The lignin was filtered, washed with hot water, and dried at 105° C. Two such experiments were carried out; the lignin from these experiments was combined and the percentages nitrogen and methoxyl in the lignin were determined. The former was determined by the Kjeldahl-Gunning-Arnold[1] method and the latter by the Kirpal and Bühn modification of the Zeisel method.[2] A weighed portion (5 grams) of this lignin was placed in one of the reaction tubes of the apparatus described by Goss and Phillips[3] and treated with 50 cc. of fuming hydrochloric acid for 24 hours and subsequently with boiling 5 per cent hydrochloric acid according to the procedure recommended by Goss and Phillips[4] for the quantitative estimation of lignin. The residual lignin was filtered on a weighed sintered-glass crucible and dried at 105° C., and the loss in weight was determined. A portion of the residual lignin was taken for analysis, and the remainder was subjected to the alternate treatment with cold fuming hydrochloric and boiling 5 per cent hydrochloric acid as already described. Five such experiments were carried out, and with the exception, of course, of Experiment No. 1, the residual lignin from the preceding experiment was used for the successive hydrolysis with cold fuming hydrochloric acid and boiling 5 per cent hydrochloric acid. The results obtained are recorded in the table.

Effect on lignin of successive treatments with fuming and dilute HCl
Anal. of Original Lignin: N, 0.66%
OCH_3, 14.15%

EXPERIMENT NO.	WT. OF LIGNIN TAKEN	WT. OF RESIDUAL LIGNIN	LOSS DUE TO HYDROLYSIS		N IN RESIDUAL LIGNIN	OCH_3 IN RESIDUAL LIGNIN
	grams	*grams*	*grams*	*per cent*	*per cent*	*per cent*
1	5.0000	4.3733	0.6267	12.53	0.65	14.18
2	3.4408	3.3202	0.1206	3.50	0.59	14.38
3	2.0640	1.9994	0.0646	3.12	0.61	14.09
4	1.1163	1.0937	0.0226	2.02	—	14.03
5	0.6413	0.6239	0.0174	2.71	—	14.18

It will be observed that the greatest loss of lignin (12.53 per cent) occurred in the first experiment. In the subsequent experiments, the losses were considerably less and varied from 2 to 3.5 per cent. The percentage nitrogen in the residual lignin was not affected by the hydrolytic treatment, thus indicating that the nitrogenous complexes associated with the lignin were broken down at approximately the same rate as the lignin itself. The figures on the percentage methoxyl are most interesting, as they indicate very definitely that the lignin itself had been hydrolyzed

[1] *Methods of Analysis, A.O.A.C.*, 1935, 25.
[2] *Ber.*, 47, 1084 (1914); *Monatsh.*, 36, 853 (1915); *This Journal*, 15, 118 (1932).
[3] *Loc. cit.*
[4] *Loc. cit.*

and that the loss suffered was not due to the removal of some impurity in the lignin preparation, unless, of course, it be assumed that in Willstätter lignin from wheat straw there is present a more hydrolyzable substance having the same percentage methoxyl as lignin itself. This is, however, extremely improbable. It is more reasonable to assume that lignin itself had suffered hydrolysis. It is conceivable, of course, that Willstätter lignin may be a mixture of closely related or isomeric lignins and that one or more of these lignins may be more susceptible to hydrolysis than the other isomers. Further investigation will be required to elucidate this point, as well as to ascertain the nature of the hydrolytic fission products from Willstätter lignin. This investigation is being continued.

SUMMARY

Willstätter lignin from wheat straw was subjected to the alternate treatment with cold fuming hydrochloric acid and boiling 5 per cent hydrochloric acid. As a result of this treatment, a portion of the lignin complex as a whole was hydrolyzed, as was evident from the fact that the percentage methoxyl in the residual lignin remained the same as in the original lignin.

DETERMINATION OF ROTENONE IN DERRIS AND CUBE

III. AN IMPROVED CRYSTALLIZATION METHOD

By HOWARD A. JONES (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.) and J. J. T. GRAHAM (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In the previous accounts of this investigation by Jones[1] and by Jones and Graham[2] the various methods proposed for the determination of rotenone in derris and cube roots by extraction and crystallization were cited, and several of them were studied and compared. On the basis of this study the following procedure is recommended for the analysis of finely powdered derris or cube root for its rotenone content:

Weigh 30 grams of the finely powdered root into a 500 cc. glass-stoppered Erlenmeyer flask. Add 300 cc. of $CHCl_3$ measured at a definite room temperature; place the flask on a shaking machine and fasten the stoppers securely. Agitate vigorously for not less than 4 hours, preferably interrupting the shaking with overnight rest. (As an alternative procedure the flask may be shaken continuously overnight.) Remove the flask from the machine and allow to cool in a refrigerator for at least an hour. Filter the mixture rapidly into a suitable flask, using a fluted paper without suction and keeping the funnel covered with a watch-glass to avoid loss from evaporation. Stopper the flask and adjust the temperature of the filtrate to that of the original $CHCl_3$.

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[1] *Ind. Eng. Chem. Anal. Ed.*, 9, 206 (1937).
[2] *Ibid.*, 10, 19 (1938).

Transfer exactly 200 cc. of this solution to a 500 cc. Pyrex Erlenmeyer flask and distil until only about 25 cc. remains in the flask. Transfer the extract to a 125 cc. Erlenmeyer flask, using CCl_4 to rinse out the 500 cc. flask. Evaporate almost to dryness on the steam bath in a current of air. Then remove the remainder of the solvent under reduced pressure, heating cautiously on the steam bath when necessary to hasten the evaporation. (The suction may be applied directly to the flask.) Dissolve the extract in 15 cc. of hot CCl_4 and again, in a similar manner, remove all the solvent. Repeat with another 10–15 cc. portion of hot CCl_4. (This treatment removes all the $CHCl_3$ from the resins.) The $CHCl_3$ extract is usually completely soluble in CCl_4. If small quantities of insoluble material are present, the purification procedure described later will eliminate them. However, if a large quantity of insoluble residue should remain when the extract is dissolved in the first portion of CCl_4, it should be filtered off and thoroughly washed with further portions of hot solvent, after which the filtered solution plus washings should be treated as described above for the removal of $CHCl_3$.

Add exactly 25 cc. of CCl_4 and heat gently to completely dissolve the extract. Cool the flask in an ice bath for several minutes and seed with a few crystals of rotenone-carbon tetrachloride solvate if necessary. Stopper the flask and swirl until crystallization is apparent. If at this stage only a small quantity of crystalline material separates, add an accurately weighed quantity of pure rotenone, estimated to be sufficient so that the final result, expressed as pure rotenone, is at least 1 gram. Then warm to effect complete solution, and again induce crystallization. At the same time prepare a saturated solution of rotenone in CCl_4 for washing. Place the flasks containing the extract and the washing solution in an ice bath capable of maintaining a temperature of 0° C. and allow to remain overnight.

After 17–18 hours in the ice bath, rapidly filter the extract through a weighed Gooch crucible fitted with a disk of filter paper, removing the flask from the ice bath only long enough to pour each fraction of extract into the crucible. Rinse the residue of crystalline material from the flask and wash under suction with sufficient of the ice-cold saturated solution (usually 10–12 cc.) to remove the excess mother liquor. Allow the crucible to remain under suction for about 5 minutes and then dry to constant weight at 40° C., which usually requires about an hour. The weight obtained is the "crude rotenone-carbon tetrachloride solvate."

Break up the contents of the crucible with a spatula, mix thoroughly, and weigh 1 gram into a 50 cc. Erlenmeyer flask. Add 10 cc. of alcohol that has previously been saturated with rotenone at room temperature, swirl the flask for a few minutes, stopper tightly, and set aside for at least 4 hours, preferably overnight, at the same temperature. Filter on a weighed Gooch crucible fitted with a disk of filter paper. Rinse the crystals from the flask and wash under suction with a solution of ethyl alcohol saturated with rotenone at the temperature of recrystallization (5–10 cc. will usually be required). Allow the crucible to remain under suction for 3–5 minutes and then dry at 105° C. to constant weight, which should be effected in 1 hour.

Multiply the weight, expressed in grams, by the weight of the crude rotenone-carbon tetrachloride solvate, and to the product add 0.07 gram, which represents the correction for rotenone held in solution in the 25 cc. of CCl_4 used in crystallization. If any pure rotenone has been added, subtract its weight from the value obtained. This gives the weight of pure rotenone contained in the aliquot of the extract, representing 20 grams of the sample.

DISCUSSION OF THE METHOD

General Notes.—Both the chloroform and the carbon tetrachloride should be of reasonably high purity and entirely free of water and acid.

The saturated solution of rotenone in carbon tetrachloride may be prepared by adding an excess of rotenone, warming to effect solution, and then keeping at 0° C. in the ice bath overnight and filtering immediately before using; or by dissolving 0.27 gram of pure rotenone in 100 cc. of carbon tetrachloride and then cooling to 0° C. for use.

In very hot weather it is advisable to weigh the flasks and contents at the beginning of the extraction and again before filtering. If there is an appreciable loss, the original weight should be restored by the addition of chloroform before the contents of the flask are filtered. It is also advisable in hot weather to cool the crucibles in a refrigerator immediately before use.

Extraction.—Methods for the extraction of rotenone from root samples were studied in Part II of this investigation. The foregoing procedure was found to be satisfactory for finely powdered roots and much shorter than other methods. However, fineness of grinding was of paramount importance whatever the method of extraction used. To obtain quantitative extraction by the proposed method, at least 95 per cent of the sample must pass a 60-mesh sieve. For rapid extraction the sample should be finer than this.

Another factor that appeared to influence the ease of extraction was the ratio of rotenone to total extractive material. In samples in which the ratio of rotenone was high, extraction was difficult. The present method will give satisfactorily complete extraction provided this ratio does not exceed 40 per cent. Fortunately derris and cube samples with a ratio higher than this are rarely encountered.

For roots in which the ratio of rotenone to total extract is higher than this, or if for any other reason there is doubt as to the completeness of extraction, an alternative procedure involving room-temperature extraction with successive lots of chloroform is suggested. The procedure is similar to that proposed except that the root is filtered from the first extract, washed, treated with fresh solvent, and again shaken. Four successive treatments with chloroform in this way are recommended. The combined extract is treated as outlined in the proposed method. The final marc may be boiled with acetone and this extract concentrated and tested by the modified Durham test[1] to determine whether any rotenone remains unextracted. In the previous work this method, although more time-consuming than the aliquot procedure, was found to give more complete extraction of those few samples having unusually high ratios of rotenone to total extract. This method also extracted coarsely ground samples more satisfactorily.

Crystallization.—In Part I of this series the factors involved in the crystallization of rotenone from extracts were studied. It was found that the nonrotenone resins markedly retarded the rate of crystallization,

[1] Jones, H. A. and Smith, C. M., *Ind. Eng. Chem. Anal. Ed.*, 5, 75 (1933).

causing low results when less than 1 gram of rotenone was present in the extract from 20 to 25 grams of root. The simplest method of overcoming this difficulty is the addition of rotenone to the extracts of roots low in rotenone. After a little experience with the method the operator will be able to decide at the time of first crystallization whether rotenone must be added, and a complete preliminary analysis will not be necessary. Care should be taken to completely dissolve the added rotenone by warming and to recrystallize, otherwise some of it may remain unsolvated.

Accuracy.—In the analyses of 31 samples of derris and cube, some containing as high as 10 per cent of rotenone, the results of the two authors never differed more than 0.4 per cent, and the average difference was only 0.2 per cent. Equally as good results should be obtained by any careful analyst.

DETERMINATION OF NICOTINE ON APPLES SPRAYED WITH NICOTINE BENTONITE

By L. N. Markwood (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

The Ralston method[1] is used in the Division of Insecticide Investigations for determining the quantity of nicotine on apples sprayed with a nicotine insecticide. In this method the apples are shaken in a closed container with a mixture of dilute sodium hydroxide solution and ethylene dichloride. The nicotine set free is taken up by the organic solvent, from which it is subsequently extracted with hydrochloric acid, then distilled from alkaline solution, and precipitated with silicotungstic acid in the usual way.[2] This method is applicable to any of the known nicotine combinations.

The work reported in this paper represents an attempt to develop a method specifically applicable to nicotine bentonite, since this product is now coming into use, and to devise a more convenient stripping process through elimination of the shaking operation. A dipping method, wherein each apple of the sample to be analyzed is separately immersed in a denicotinizing solution contained in a beaker, seemed to be the simplest procedure.

Immersion in a solution of sodium hydroxide does, of course, strip the nicotine from the fruit, but the use of an open beaker involves likelihood of loss of nicotine. Although the use of an acid seemed to be appropriate to prevent escape of nicotine, immersion in an acid solution (hydrochloric acid) was ineffective, as the nicotine bentonite only partially released its nicotine. Experiments were then conducted to learn whether the nicotine could be brought into solution by base-exchange with metallic salts in

[1] Unpublished.
[2] *Methods of Analysis, A.O.A.C.*, 1935, 60.

acid solution. Salts of sodium, barium, calcium, magnesium, aluminum, and lead were tested, but none of them gave even approximately complete recovery of the nicotine. The barium salt, for example, removed only 40 per cent, which is not greatly in excess of that removed by hydrochloric acid alone (22 per cent). Organic bases were then tried, and these gave better results. β-naphthylamine was partly satisfactory in that it seemed to leave little if any nicotine in the insoluble form, but owing to its volatility with steam it interfered with the subsequent silicotungstic acid precipitation.

Brucine presented no such disadvantages. It is not volatile with steam and even when the acid solution containing it is made alkaline the precipitated base formed in the cold changes on heating into a globular form, which does not interfere with the smoothness of the distillation. As the analytical data obtained with brucine were considered satisfactory, the method formulated and described herein was based upon the replacement of nicotine by brucine.

BASE-EXCHANGE EXPERIMENTS WITH BRUCINE

A quantity of nicotine bentonite was prepared from nicotine and bentonite in the presence of hydrochloric acid (5.1 grams of nicotine to 95 grams of bentonite) practically as described by C. R. Smith.[1] This product, when dried, powdered, run through a 150-mesh screen, and analyzed by the silicotungstic acid method was found to contain 5.00 per cent of nicotine. A suspension of 0.2000 gram of this nicotine bentonite in 200 cc. of water containing 10 cc. of hydrochloric acid (1+4), plus a weighed quantity of brucine, was boiled for 4 minutes. The soluble and insoluble parts were then separated by filtration through a Gooch crucible, and the residue was washed well. The filtrate was boiled to a volume of about 75 cc., treated with a slight excess of sodium hydroxide, and distilled for its nicotine content. Similarly, the insoluble residue was analyzed for nicotine.

TABLE 1.—*Effectiveness of brucine in removing nicotine from nicotine bentonite*

BRUCINE TAKEN	NICOTINE IN SOLUTION	NICOTINE IN RESIDUE	TOTAL NICOTINE RECOVERED	NICOTINE REMOVED FROM NICOTINE BENTONITE*
grams	*per cent*	*per cent*	*per cent*	*per cent*
2.0	84.4	7.1	91.5	92.9
2.5	84.7	6.8	91.5	93.2
None	32.8	64.3	97.1	35.7

* Obtained by deducting the nicotine in the residue from 100 per cent.

The data in Table 1 show the effectiveness of brucine in removing nicotine from nicotine bentonite.

[1] *J. Am. Chem. Soc.*, 56, 1561 (1934).

The results (Table 1) show that in no case was the total original quantity of nicotine recovered. When brucine was used, the quantity recovered in both soluble and insoluble fractions was 91.5 per cent of that originally present, a loss of 8.5 per cent. Even when no brucine was present there was a loss of about 3 per cent. This loss appears to be due to decomposition (air oxidation) of nicotine during the filtration of the hot acid solution. The loss probably occurs in the dissolved nicotine, which point is borne out by the fact that the losses are roughly proportional to the percentage of nicotine in solution. Since the loss occurs after the nicotine has been removed from its bentonite combination the efficiency of removal of nicotine (as distinct from its recovery) is properly determined by deducting the amount in the residue from 100 per cent. It is shown that brucine removes about 93 per cent of the total nicotine, a result distinctly superior to that obtained with hydrochloric acid alone. Little advantage is gained by using more than 2 grams of brucine.

BRUCINE TREATMENT OF APPLES

The process thus developed seemed to be suitable for the determination of nicotine residues on apples. For this purpose the recovery of nicotine and not simply its removal is important, but the conditions in stripping an apple are somewhat different from those described for nicotine bentonite powder, and are likely to favor a greater proportional recovery of nicotine. In the first place, the filmy deposit ordinarily found on an apple gives greater surface exposure than does a powder of 150-mesh size. Secondly, no filtration of the hot acid solution is required, and hence less loss by oxidation should result.

Before this process was applied to apples, consideration was given to the desirability of including an agent in the mixture to wet the surface of the apple and to aid in removing the wax. Several commercial products were tried, and the one found to be most stable in the acid and alkaline media employed in the test was selected for use. It had no effect on the amount of nicotine removed from the bentonite powder.

Fifty Stayman Winesap apples (picked during the growing season) of approximately uniform size and sprayed with the greatest uniformity possible by laboratory technic* were divided into lots of 10. Four lots were treated by immersion in boiling stripping solutions. Each of the solutions contained 180 cc. of water and 20 cc. of the dilute hydrochloric acid, and in addition for Lot 2, 0.25 gram of wetting agent, for Lot 3, 2 grams of brucine, and for Lot 4, both 0.25 gram of wetting agent and 2 grams of brucine. Each apple was held 4 minutes in the solution and then rinsed off in another beaker. A noticeable result of treatment with brucine is the building up of deposit, when the original flat film increases in thickness and stands out in marked relief. The acid extracts were con-

* The spraying was done by Dr. E. H. Siegler, of the Division of Fruit Insect Investigations.

centrated on the steam bath to about 75 cc. before the alkaline distillation, and the usual precipitation with silicotungstic acid followed. The fifth lot was treated by the Ralston method modified by the substitution of toluene for ethylene dichloride, the supply of which was temporarily depleted.

TABLE 2.—*Nicotine coverage on apples by acid-immersion*

LOT	METHOD	NICOTINE FOUND	RELATIVE AMOUNTS FOUND
		mg.	*per cent*
	Hydrochloric acid solution with—		
1	No wetting agent or brucine	3.59	61
2	Wetting agent	3.59	61
3	Brucine	5.87	100
4	Wetting agent and brucine	5.87	100
5	Modified Ralston	4.79	82

The results in Table 2 show that when brucine was used the amount found was highest. The wetting agent is apparently unnecessary. The brucine method gave a higher amount than did the Ralston method.

A second group of apples, also approximately uniform in size and sprayed with uniform care, was divided into 6 lots of 10 each. The nicotine load of each lot was determined. The stripping solution for Lots 1 and 2 consisted of 180 cc. of water, 20 cc. of dilute hydrochloric acid, and 2 grams of brucine; for Lots 3 and 4, 0.25 gram of wetting agent was added. Lots 5 and 6 were treated by the original Ralston method, with ethylene dichloride and sodium hydroxide as the solvents.

TABLE 3.—*Nicotine coverage on apples by the brucine and Ralston methods supplementary tests*

METHOD	LOT	WEIGHT OF LOT	NICOTINE FOUND		RELATIVE AMOUNTS FOUND	
			PER LOT	PER KILOGRAM	PER LOT	PER KILOGRAM
		grams	*mg.*	*mg.*	*per cent*	*per cent*
Brucine—						
Without wetting agent	1	866	5.29	6.12		
	2	867	5.30	6.12		
		Average	5.30	6.12	100	100
With wetting agent	3	858	5.23	6.11		
	4	873	5.18	5.93		
		Average	5.21	6.02	98	98
Ralston's original	5	830	4.46	5.37		
	6	861	4.53	5.27		
		Average	4.50	5.32	85	87

The results shown in Table 3 substantiate the previous findings. The quantity of nicotine found by the Ralston method is appreciably lower than that found by the brucine method. Since every effort was made to apply uniform coverage to each apple, the methods may be compared either on the basis of a lot of 10 apples or on the kilogram basis. Duplicates by each method are in fair agreement. The addition of the wetting agent gave no apparent advantage. A stripping solution containing 2 grams of brucine, 20 cc. of hydrochloric acid (1+4), and 180 cc. of water is the one indicated for adoption. If necessary to obtain complete immersion of a large apple, an increased volume of water may be taken. The apple is kept submerged either by a pointed glass rod or a three pronged device constructed of glass tubing.

Brucine seems to fulfil the requirements of this work satisfactorily and is a relatively inexpensive reagent (present price about 10 cents per ounce).

BOOK REVIEWS

Potash Deficiency Symptoms. By OSKAR ECKSTEIN, ALBERT BRUNO, and J. W. TURRENTINE with the collaboration of G. H. COWIE and G. N. HOFFER. Verlagsgesellschaft für Ackerbau M.B.H. Berlin S.W. 11. 1937.

The book represents the cooperative efforts of the German, French, and American potash industries. The authors have had extensive experience in dealing with fertilizer problems, especially with those relating to potash. The presentation of the subject matter including the index, table of contents, and a foreword by Gabriel Bertrand in three languages, German, French, and English renders the work readily available to an extensive public.

The first part deals with the general symptoms of potash deficiency, including the effect on the leaf, root, blossom, fruit, appearance of the plant, and the inner structure of the plant; the secondary effects of potash deficiency such as the influence on resistance to plant diseases, insect pests, and adverse climatic conditions; relation of market value of crop to potash deficiency and the pathology of potash deficiency.

The second part discusses potash symptoms of various cultivated crops. The section contributed by Hoffer deals with the recognition of symptoms of appearance, lodging, and root systems, hereditary factors in diagnosis of potash deficiency symptoms, and the use of chemical tests in the confirmation of deficiency symptoms in corn and other cereals. Cowie contributes the section on fruit trees, which deals with the development of "leaf scorch," nitrogen and potash balance, and effect on yield, size, and color of fruit.

Forty-one photographs and 54 color plates with descriptive legends illustrate the symptoms of potash deficiency in crops, and a colored chart shows the amount of N, P_2O_5, and K_2O removed by various crops. The bibliography contains 209 titles.

The book is an excellent presentation of the characteristic symptoms of potash deficiencies.—H. R. KRAYBILL.

Selected Topics in Colloid Chemistry. By ROSS AIKEN GORTNER. 169 pp., 35 figures. Cornell University Press, Ithaca, N. Y., 1937. Price $2.50.

This book covers the content of a series of lectures delivered in the Department of Chemistry of Cornell University during the first semester of the academic year 1935–6, at which time the author held the George Fisher Baker Non-resident Lectureship in Chemistry. The author is convinced that in the study of the phenomena characteristic of colloidal systems we shall find clues that will enable us to understand many of the reactions characteristic of living organisms and life processes.

The first three chapters, and, to a certain extent, the fourth, serve to give the necessary background for the remaining portion of the book. Chapter I is an historical treatment of the work of Thomas Graham in laying the foundations of colloid chemistry. Chapter II delimits the field of colloid chemistry, while Chapter III presents some of the basic concepts and methods of colloid chemistry. Chapter IV treats of the properties of colloidal systems such as Brownian movement, dialysis, ultra-filtration and viscosity, and plasticity. Much of the illustrative material is taken from the author's own work in the biochemical field.

Chapter V gives an excellent treatment of the subject of electrokinetics, a field in which the author and his students have made many important contributions. Chapter VI treats surface phenomena, while Chapter VII deals with the subject of adsorption.

Chapter VIII, which comprises almost one-fourth of the book, summarizes the author's contributions in the water relations of biocolloids and evaluates the con-

tributions of other authors. One cannot read this chapter and fail to realize the importance of water relations in biocolloids, nor can he fail to realize the need for further research.

The book as a whole is stimulating, easy to read, and rather free from errors. Workers in the biochemical field will find it well worth reading. A non-technical lecture on scientific genealogy, which is included with the other lectures, gives an interesting glimpse of the author's personality.—LYLE T. ALEXANDER.

Practical Methods in Biochemistry. By FREDERICK C. KOCH, Professor of Biochemistry, University of Chicago. Second edition. William Wood & Co. 1937; ix+302 pp. Price $2.25.

This is a thoroughly revised and modernized version of the first edition, which was published in 1934, and should prove of value in teaching an elementary laboratory course to medical or pre-medical students, for whom it has been designed.

Since this manual is intended primary as a practical companion to Professor A. P. Mathews' textbook on "Physiological Chemistry," the subject matter is presented in the same order as presented in the latter textbook, with over one-third of the pages devoted to quantitative methods for blood and urine analyses.

The descriptions of the manner of carrying out the various qualitative tests on carbohydrates, fats, and proteins are accompanied by concise notes explaining the limitations of the tests and some of the chemistry involved, while each of the descriptions of the quantitative analytical methods is preceded by a rather complete explanation of the principles involved. This should prove a convenient feature in aiding the student to correlate the theoretical and practical aspects of the subject while actually working with the latter in the laboratory.

The 54 page appendix contains general laboratory instructions, as well as instructions for the preparation of solutions and reagents, which are sufficiently detailed to enable the student, himself, to prepare these as needed.

The value of the manual to the medical student might have been further enhanced by utilizing some of the space, devoted to preparation of solutions and reagents, for the presentation of more work on the chemistry of the digestive tract, proteins, carbohydrates, and of fats.—C. S. MYERS.

The Structure and Composition of Foods. By ANDREW L. WINTON and KATE BARBER WINTON. Volume III. Milk (including human), Butter, Cheese, Ice Cream, Eggs, Meat, Meat Extracts, Gelatin, Animal Fats, Poultry, Fish, Shellfish. 524 pp., illustrated. John W. Wiley & Sons, Inc., New York, 1937. Price $8.00.

It is with a little disappointment, perhaps, that one views this third volume of this series. Experience with Volumes I and II had demonstrated the value of this work to all interested in the structure and composition of foods and had led to an anticipation of its completion as originally contemplated in Volume III. It is most likely that this volume, dealing with foods of animal origin, has become available sooner than would otherwise have been possible had its publication awaited inclusion of the remaining untreated foods. A review of its context shows that, like its precedessors, it will be found to be of the utmost usefulness, and its early appearance compensates for the initial disappointment.

The authors have continued in their painstaking and thorough manner in compiling the essential information from the world's literature, and have followed their established style of presentation. Unlike the foods dealt with in Volumes I and II, those treated here do not offer much opportunity for discussion of microscopic methods of examination. In Part 3, on meat and fish, the usual proximate composi-

tion and constituent analyses have been supplemented by results of investigations reported in recent years involving detailed descriptions of proteins, fats, carbohydrates, meat bases, and phosphorus-organic compounds involved in muscular effort. As with all works so comprehensive, even such care as it is evident these authors have exercised cannot always prevent occasional errors or misstatements from creeping in. While no attempt will be made to list those that have been noticed, the failure to state the fat percentage required by the United States standards for cheese as being on the moisture-free basis is an especially noticeable one. This volume is a welcome addition to the series, the completion of which will be regarded by all workers in the many fields of technology and science of foods as an invaluable contribution to the world's knowledge of food composition and structure.—HENRY A. LEPPER.

Volumetric Analysis. By A. J. MEE. Published in Great Britain in 1937. American agent and distributor: Chemical Publishing Company of New York, Inc. 148 Lafayette St., New York. Price $3.50.

The preface states that the book aims to prepare students to apply volumetric analysis to the solution of practical chemical problems. It is well fitted to do just that.

Some of the more intricate applications of volumetric analysis are missing. This omission, however, can not be criticized because it is in line with the expressed intention of the author of including only such methods as can be relied on to give accurate results in the hands of relatively inexperienced analysts. Yet the book is not without value to the more experienced man. Attention also has been given to up-to-date methods, including the use of ceric sulfate and adsorption and oxidation-reduction indicators.

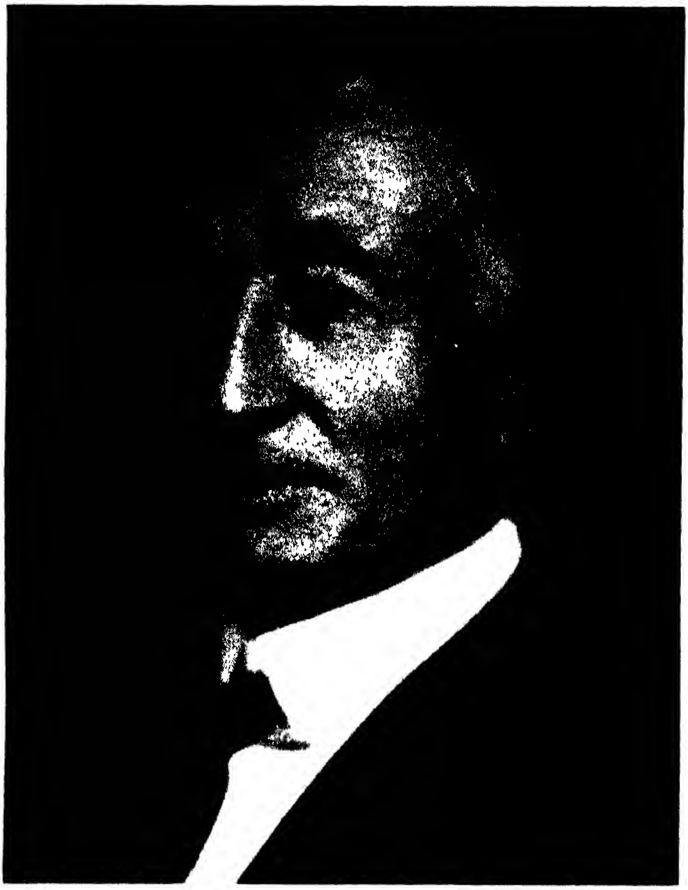
The first chapter gives a concisely written introduction to the subject, stressing many points that both the beginner and the experienced analyst may profit by noting. The paragraphs dealing with preparation and storage of standard solutions and with the accuracy of volumetric methods are particularly enjoyable as well as instructive.

Ten chapters dealing with subjects such as Acidimetry and Alkalimetry, Use of Ceric Sulfate, Iodimetry, Precipitation Methods, are followed by three appendices dealing with automatic burette and pipette, indicators, and list of materials and solutions. There is a general index as well as a special index to some 200 problems and, finally, a table of four-place logarithms.

Each chapter gives a short treatise on the necessary theory and a number of experiments. These experiments are well chosen, and the directions are to the point, yet free from burdensome detail.

The book should be a suitable laboratory guide. It anticipates and answers many questions that the beginner is apt to ask the teacher.

If anything, the book offers a more complete course in experimental volumetric analysis than the student ordinarily has time to assimilate. Some modern teachers may wish for more theoretical treatment here and there, but on the whole the balance is well struck between theory and experimental applications.—DAN DAHLE.



Richard Newman Brackett, 1863–1937

RICHARD NEWMAN BRACKETT

In the demise of Doctor Richard Newman Brackett, this Association suffered an irreparable loss. His counsel, his familiarity with precedent and orderly procedure, his knowledge of the development of the official methods, his sagacity, and his unerring judicial attitude were recognized, appreciated, and utilized in full measure. The devoted and effective service rendered by Doctor Brackett eventuated in many pages of type, dispersed throughout the records of the Association during the twenty-one years of his participation in its activities. Of that service he wrote: "I count those twenty-one years as among the most interesting and inspiring of my life." Of him the lovable and lamented Benjamin B. Ross wrote for the American Chemical Society: "To enjoy so large a measure of the esteem and confidence of one's colleagues and coworkers as to be called on times without number to discharge tasks and assignments that demand thoroughness and efficiency should occasion a feeling of satisfaction and pride in the man whose friends so regard him. Such a man is Richard Newman Brackett . . . ."

Doctor Brackett was the son of a Presbyterian minister, who was a native of Massachusetts, and of Louisa Theresa Newman, whose parents had moved from Baltimore to South Carolina. He was born at the home of his maternal grandfather near Columbia, Richland County, South Carolina, September 14, 1863, and died in his campus home at Clemson College, November 27, 1937. He attended private schools in Charleston, 1870-1878, and at the age of 15 entered the sub-freshman class of Davidson College, where he was a classmate of the late Doctor W.A.Withers and whence he graduated in 1883. In September of that year he enrolled as a graduate student at Johns Hopkins University, with a major in chemistry under Remsen, and minors in mineralogy, dynamic geology, and microscopic petrography. Upon attainment of his doctorate, in 1887, he became Chief Chemist to the Geological Survey of Arkansas. During his four years of service there, he and his colleague, J. Travers Williams, discovered two minerals, newtonite and rectorite, of the kaolinite group.

In November, 1891, he became a member of the first faculty of Clemson College, and survived all other members of that group, serving the institution continuously for forty-six years. During that period he served as Associate Professor, 1891–1910; Acting Professor of Chemistry, Acting Director of the Department of Chemistry, and Acting Chief Chemist of the Fertilizer Division, 1910–1911; Professor and Director of the Department of Chemistry and Chief Chemist of the Fertilizer Division, 1911–1933; and Professor of Chemistry and Curator of the Chemical Library, 1933–1937. From 1920 to 1928, he was also Chemist of the South Carolina Experiment Station.

He was married in 1889 to Bessie Brandon Craig, daughter of a Presbyterian minister of Atlanta, Georgia. From that union were born three children: Richard Brandon, who died at the age of four years; Helen, now Mrs. Franklin Turner Waddill, of Cheraw, South Carolina; and Newton Craig, a practising physician of Pickens, South Carolina.

Doctor Brackett became a member of the American Association for the Advancement of Science in 1887, a Fellow in 1891, and an Emeritus Member in 1936. He joined the American Chemical Society in 1894, and served as Chairman of the South Carolina Section. He was also

a member of the South Carolina Academy of Science, and was its President in 1930–1931. He was an ardent Mason, and served as Master of his lodge.

In his affiliation with the Association of Official Agricultural Chemists, Doctor Brackett contributed liberally and effectively. During his twenty-one years of membership in and service to that organization, he missed only one meeting, and that because of illness. At the 29th meeting, in 1912, he presented a comparative study of the official method for the determination of potash and a proposed modification, and at that meeting he was made Referee on Nitrogen. At the 30th meeting, 1913, he advanced the proposal that the Association assume responsibility for the publication of its proceedings and analytical methods. The 33rd meeting, 1916, was conducted under his presidency, and at the 1917 meeting he presented a gavel made of wood from the historic Calhoun homestead at Clemson. He was made Referee on Fertilizers at the 35th meeting, 1919, and served as such for five years. He gave reports on fertilizers at the 36th, 37th, 38th, and 39th meetings. At the 41st meeting, 1925, he became a member of the Committee on Definitions of Fertilizers, and served thereon through 1932. During the period 1926–1932 he also served as a member of the Committee for Sampling of Fertilizers, and in 1928 contributed a bibliography on that subject. Of particular value is his unpublished history and bibliography of the phosphate literature, a treatise that the Executive Committee had asked, in 1937, that he bring to date for publication by the Association.

Coincident with the announcement of his retirement and his attendance at the 1932 meeting of the Association, Doctor Brackett was accorded a most unusual manifestation of the esteem in which he was held. A testimonial dinner in his honor at the Cosmos Club afforded his colleagues the opportunity to express appreciation of his lovable disposition and unselfish character, to voice admiration of his usefulness, and to extend their recognition of his many scientific contributions. Appropriate references to Brackett, the efficient chemist, the inspirational teacher, and the ideal citizen, were given by a number of his coworkers. As a memento of the occasion, he was presented with a beautiful piece of silver.

Although he contributed much as administrator and as regulatory official, and in the work and councils of the Association, Doctor Brackett's preeminent achievement was character-building among the many students privileged to enjoy his tutelage. His unbounded love for teaching was his dominant characteristic. In acknowledgment of a letter from the Secretary of Agriculture, who requested his presence as an honored guest at the "Pioneer's Program" in Washington, November, 1937, Doctor Brackett wrote: "Of course, helping thousands of young men prepare for life, watching their careers after graduation (Clemson boys have 'made good' all over the world), and seeing and taking a modest part in the growth of a great college, have been the chief interests and inspirations of my life."

His passionate devotion to the opportunity afforded by his class room and his laboratory to inculcate his concepts of integrity and usefulness has left indelible impress upon many who freely and proudly acknowledge their indebtedness to him. His orthodox philosophy, his keen sense of humor, his modest self-effacement, and his high ideals did much to mold character and to stimulate and inspire those who came under his benign influence. We have known many who learned

from him, whom they effectionately and respectfully dubbed "Dicky" Brackett, an appellative that pleased and thrilled him. Without exception, those former students have spoken with pride of their associations with him, have recounted with smiles and laughter anecdotes of their days as his pupils, and have gratefully expressed their appreciation of his continued interest and practical helpfulness after their graduation. His students knew that his daily life and actions were motivated by his deep-seated religious convictions and by his appreciation of the obligations that he felt were corollary to his position. As indicative of the sustained esteem of those who had known him in their hypercritical days as students, Doctor Brackett was elected to honorary membership in the Clemson Alumni Corporation.

His home, a place of charm, of culture, and of refined contentment, was a Mecca to his students and to many of us who were so fortunate as to be guests therein. The genial warmth and spontaneous hospitality extended by him and by his gracious wife will be remembered by many as experiences most pleasant.

A former associate, a Past President of the Association of Official Agricultural Chemists, wrote: "Doctor Brackett lived an exemplary life, was a devoted husband and father, an active church worker and took an active interest in the welfare of his fellowmen." He served the Fort Hill Presbyterian Church fourteen years as Deacon, twenty-two years as Elder, many years as Clerk of The Session, and as Sunday School teacher. He was a patron of the Clemson Young Men's Christian Association, and was largely instrumental in obtaining the erection of a splendid building for that organization.

To this writer, he was the personification of the Christian Gentleman, in the full implication of that term. Never, during our thirty years of privileged association and friendship with him, did we hear him speak an unkind work; never did we hear him utter a phrase that could not be repeated in the most sacred precinct or that could be considered as unseemly by even the most devout.

Upon the death of each of his friends and confreres of many years, Doctors Withers and Frear, he recorded his appreciation of their worth in words and expressions that are equally true of himself. To these we would give echo, in quoting and applying them as expressions more appropriate than any that we might offer. Of his classmate, Doctor Withers, he wrote: "As a man, (he) was characterized by modesty and integrity. He was a true and loyal friend, a lover of home and family, and from early youth an earnest, conscientious Christian. His sympathies and interests were broad, as shown by his diligence as teacher and research worker, his keen and active interest in civic affairs, and his devotion to the things of the Spirit."The concluding paragraph of his tribute to Doctor Frear read, "When the summons came he met death, of which he had no fear, 'like one who wraps the drapery of his couch about him and lies down to pleasant dreams'." These expressions of his regard for his two friends epitomize the esteem in which Doctor Brackett was held by all who knew him.

To all of us his demise is a personal loss that words cannot adequately express. He was truly a benign influence, an inspiration, a character most worthy of emulation, one who deserved the praise accorded by him to another: He "left the world better for having lived in it."

WALTER HOGE MACINTIRE

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. Sale (U. S. Food and Drug Administration, Washington, D. C.), Referee

A survey of the literature published during the past year on analytical methods for alcoholic beverages showed considerable activity on the part of workers in this field. In a summary of these activities from the standpoint of enforcement of laws dealing with the control of beverages, the laboratory work on the identification and determination of the characterizing ingredients of such beverages is naturally of greatest concern to the members of this Association. One of the most important of these constituents is the higher alcohols, on the determination of which a number of papers were published during the past year.

Penniman, Smith, and Lawski[1] revived the colorimetric procedure for higher alcohols, which depends upon the Komarowsky reaction, and improved the technic to give greater dependability. The method, however, is still subject to the disadvantage that the best results are obtained by the use of a standard, a mixture of higher alcohols having the same composition as that of the sample.

Leitle[2] recommends a quick method for fusel oil, depending upon the change in refractive index of an immiscible solvent used to extract the higher alcohols from whiskey.

Clavera and Martin[3] made a study of the effect of methyl alcohol upon the determination of higher alcohols.

Möhler,[4] with his co-workers Hämmerle and Polya, added three papers to his series on flavoring constituents of cherry brandy.

Work done on the determination of alcohol in beverages was reported by several workers. Pidal[5] studied the distillation of alcohol-water mixtures, Luckow[6] the contraction occurring when the two liquids are mixed and the effect of extract content[7] on the determination of alcohol by the hydrometer, while Joslyn, Marsh, and Fessler[8] made an extensive study comparing various physical methods for the determination of alcohol.

A study of various methods for the chemical determination of alcohol was reported by Fabre and Bremond,[9] who came to the conclusion that an oxidation method should be used when results are desired within 0.1 per cent of the true alcohol content.

Numerous other papers on alcoholic beverages were published during

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[1] *Ind. Eng. Chem. Anal Ed.*, **9**, 91 (1937).
[2] *Z. Unters. Lebensm.*, **72**, 351 (1936).
[3] *Ann. soc. espan fis. chim.*, **34**, 507 (1936)
[4] *Z Unters. Lebensm*, **72**, 504 (1936); **73**, 171 (1937); *Mitt. Lebensm Hyg*, **27**, 40 (1936)
[5] *Bull. assoc. chim.*, **54**, 489 (1937).
[6] *Wein und Rebe*, **18**, 173 (1936).
[7] *Brennerei-Ztg.*, **52**, 161 (1935).
[8] *This Journal*, **20**, 116 (1937).
[9] *Congr. intern. tech. chim. ind. agr. 4th Congr., Brussels*, 1935, Vol. **3**, p. 367.

the year, but as they do not deal directly with analytical methods they will not be reviewed at this time. This bibliography is filed in the Beverage Section of the Food and Drug Administration, South Building of the U. S. Department of Agriculture, Room 4171, where it may be consulted.

Before the reports were given in the Alcoholic Beverage Section, W. V. Linder, of the Alcohol Tax Unit, Bureau of Internal Revenue, reviewed the work of that Unit in regard to analyzing distilled spirits and fermented beverages "to distinguish tax-paid distilled spirits from distilled spirits not tax-paid."

No formal report on malt beverages, extracts and sirups, and brewing materials was given by the associate referee.

No report on disastatic activity of malt was given by the associate referee.

REPORT ON PROTEOLYTIC ACTIVITY OF MALT

By Stephen Laufer (Schwarz Laboratories, Inc., New York City), *Associate Referee*

Two papers published this year dealing with proteolysis and proteolytic activity of barley malt deserve attention. Kolbach and Simon[1] demonstrated that the proteolysis occurring during mashing is mainly due to the action of the insoluble proteolytic enzymes present in malt, while the soluble enzymes exert only a small influence in that respect. Lhotsky and Vik,[2] on the other hand, using the Idoux method[3] in a study of the changes occurring in the proleolytic activity of malt in the course of malting, found that this activity increases considerably during germination but is reduced markedly on kilning. Pale malts of the Pilsener type gave the highest activity, whereas those of the Munich type showed comparatively lower values.

In continuing the studies initiated last year on the proteolytic activity of malt,[4] the Associate Referee concentrated his efforts on the viscometric method of measuring the activity of malt infusions. Following the view expressed in the previous paper with regard to this method, he adopted several modifications. A new viscometer was designed, having an outflow time of 40–50 seconds for 10 cc. of water at 20° C., and thus was insured a faster flow and less possibility of error due to particles coagulating in the course of making the determinations. The reaction temperature was changed from 40° to 34° C. to prevent thermal effects.

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[1] *Wochschr. Brau*, **54**, 1 (1937).
[2] *Ber. Inst. Gärungsind. tech Hochschule Brno.*, **2**, 125 (1937).
[3] *Brasserie et malterie*, **23**, 122 (1933).
[4] *This Journal*, **20**, 307 (1937).

A standard solution of pepsin (1:10,000 strength) was used to determine the reproducibility of results obtained when errors resulting from the method of extracting the malts were eliminated. For this purpose, 5 cc. of a pepsin solution (50 mg. of pepsin per 100 cc.) was mixed with 20 cc. of a 3.75 per cent gelatine solution; from this mixture 5 cc. (containing 0.5 mg. of pepsin per cc.) was pipetted into the viscometer. The following results were obtained:

Pepsin Concentration	Time Required to Reach 10% change
mg./cc.	*minutes*
0.5	61
0.5	61.5
0.5	61

The results are in good agreement.

Preliminary tests indicated that when a temperature of 34° C. was used the time necessary to reach a 10 per cent change in viscosity was considerably lengthened. For this reason it was decided to mix 10 cc. of the filtered malt infusion with 10 cc. of a 6 per cent buffered (*p*H 5.0) gelatin solution. The resulting enzyme concentration was five times that employed in the former tests, although the substrate concentration remained the same, namely 3 per cent. After 50 grams of malt had been extracted with 200 grams of water for 2 hours at 20° C., the first 35 cc. of the filtrate was found to be turbid. This portion was discarded. However, even this procedure gave inconsistent results, probably owing to variation in the amount of final drippings collected for several extractions of the same malt. The final procedure adopted was as follows: 50 grams of malt was extracted with 200 grams of water at 20° C. for 2 hours. The infusion was then filtered. The first 35 cc. was put aside, the second 35 cc. was collected and used for the viscosity test, and the remainder was mixed with the first 35 cc. and used for determining the specific gravity of the infusion. For the viscosity measurement 35 cc. of the malt infusion and 10 cc. of the 6 per cent gelatin solution were attemperated for 15 minutes at 37.5° C. At the end of this time 10 cc. of the malt infusion was added to the 10 cc. of gelatin solution and thoroughly mixed, and 10 cc. of the resulting mixture was immediately pipetted into the viscometer, which had been previously attemperated for 15 minutes in a continuously stirred glass water bath held at 34° C. (±0.02°). The viscosity determinations were carried out and the curves drawn as described in the first paper, except that only the semi-logarithmic curves were employed, as they proved to fit the points obtained better than did the plain graphs and therefore produced more reliable initial readings.

Four barley malts, two domestic 6-rowed of the Manchuria type, and two 2-rowed imported were used in these tests. In addition to the usual method of extracting for 2 hours at 20° C., infusions were prepared for

one hour at 20° C., one hour at 30° C., and one hour at 40° C. Regular laboratory mashes by the A.S.B.C. or A.O.A.C. method were also made and tested. Two malts were extracted for two hours at 20° C. with a 2 per cent sodium chloride solution to determine the effect of salt on the enzyme extraction. The results of these tests are given in Table 1.

Extraction for one hour at 20° C. gave slightly lower or about the same values as did two hours' extraction at the same temperature. For three out of the four malts extraction for 1 hour at 30° C. resulted in higher enzymic activity than did 2 hours' extraction at 20° C. With one exception, No. 3, the malt infusions tested showed a definite decline in activity when extracted for 1 hour at 40° C. From these tests it appeared

TABLE 1.—*Viscosity tests with malt infusions prepared under various extraction conditions*

MALT	EXTRACTION FOR—					REGULAR LABORATORY MASH
	2 HOURS AT 20° C.	1 HOUR AT 20° C.	1 HOUR AT 30° C.	1 HOUR AT 40° C.	2 HOURS AT 20° C. WITH 2% NaCl SOLUTION	
	Time for 10% change (minutes)					
1. Domestic, 6-rowed	37.5 37.0	37.5	36	42.5	23.5	No change
2. Domestic, 6-rowed	32.0 33.5		33.5	44.0		No change
3. Foreign, 2-rowed	67.0 68.5	69.5	62.0	59.5	54.0	No change
4. Foreign, 2-rowed	39.5 38 5	41.0	34.5	40		No change

to be advisable to retain the former method of extraction (2 hours at 20° C.). Two malt infusions prepared with a 2 per cent sodium chloride solution showed a marked increase in proteolytic activity, a phenomenon that has also been observed with diastase extractions. It is of interest to note that the worts from regular laboratory mashes exhibited a complete destruction of proteolytic activity as measured by the viscometric method. These results confirm those obtained by Kolbach and Simon,[1] who tested the proteolytic activity of worts from laboratory mashes by increase in permanently soluble nitrogen, using edestin as a substrate.

Table 2 presents the proteolytic activity units of the malts tested and the percentage deviation between two determinations for each malt. The units under Column 1 were computed, as suggested in the previous paper, on the basis that under specified conditions one unit of proteolytic activity is capable of producing a 10 per cent change in viscosity in 10 minutes. However, in the former tests viscosity measurements were run

[1] *Wochschr. Brau*, 53, 297 (1936).

at 40° C. as compared to 34° C. in the present method. For this reason the units in Column 1 are low compared to the values previously published. In order to raise these values somewhat, it is suggested that the definition for one proteolytic activity unit be tentatively revised to indicate that it is capable of producing a 10 per cent change in 40 minutes. For example, malt No. 1, domestic 6-rowed, required 37.5 minutes for a 10

TABLE 2.—*Proteolytic activity of malt infusions extracted for 2 hours at 20° C.*

MALT	DRY MALT CORRESPONDING TO 1 CC. OF THE INFUSION	10% CHANGE	1. P.A. UNITS IN 100 G. OF DRY MALT—OLD CALCULATION	2. P.A. UNITS IN 100 G. OF DRY MALT—NEW CALCULATION	DEVIATION
	grams	*minutes*			*per cent*
1. Domestic, 6-rowed	0.2258	37.5	23.9	95.7	1.4
		37.0	23.6	94.5	
2. Domestic, 6-rowed	0.2267	32.0	27.6	110.3	4.0
		33.5	26.3	105.4	
3. Foreign, 2-rowed	0.2239	67.0	13.3	53.3	2.2
		68.5	13.0	52.2	
4. Foreign, 2-rowed	0.2263	39.5	22.3	89.7	2.6
		38.5	23.0	91.8	

per cent change when 5 cc. of its infusion, corresponding to 0.2258×5 grams of dry malt, was allowed to act on the gelatin solution. This process indicates 40/37.5 units in 0.2258×5 grams, and in 100 grams of dry malt $(40/37.5 \times 100)/(5 \times 0.2258) = 95.7$ units. The revised units are given under Column 2. The maximum deviation observed for the four malts tested was 4 per cent, which is to be regarded as satisfactory.

TABLE 3.—*Proteolytic activity by the viscosity method of infusions prepared from wheat malt, wheat flour, and barley*

CHANGE IN VISCOSITY	WHEAT MALT	WHEAT FLOUR NO. 1	WHEAT FLOUR NO. 2	WISCONSIN BARLEY	CALIFORNIA BARLEY
per cent			*minutes*		
10	34				
5	15.5	110		75.5	54
2	6.5	34	58.5		

Preliminary tests were also made with one wheat malt, two wheat flours and two barleys. The values obtained, expressed in terms of time required to produce a definite change in viscosity, are presented in Table 3. The results indicate that with slight modifications the method can be adapted for determining the proteolytic activity of infusions from these materials. It might become necessary either to carry the change in viscosity up to 5 per cent only, or to use a stronger enzyme concentration in order to obtain a 10 per cent change. The wheat malt tested exhibited

about the same proteolytic power as did strongly active barley malts. When compared under 5 per cent change with the activity of the wheat malt, wheat flour No. 1, the Wisconsin barley, and the California barley tested appear about 7.1, 4.9, and 3.5 times weaker in proteolytic strength, respectively.

It is recommended[1] that the method presented here and the edestin titration method be further studied in connection with a proper mash proteolysis procedure with a view to developing one or more procedures for the collaborative testing of proteolytic activity in malt that would be of practical significance to the industries concerned.

REPORT ON MALT EXTRACT IN MALT

By E. A. SIEBEL, (8 South Dearborn Street, Chicago, Ill.), *Associate Referee*

The Associate Referee on Extract in Malt has cooperated to the fullest extent with the American Society of Brewing Chemists in evolving the present methods of malt analysis, and earnestly recommends that the procedure presented in the 1937 issue of the A.S.B.C. methods be followed.

In the meantime, further progress is being made in the study of extracts that have not been definitely investigated, and these results will also be reported through the same organization.

The methods for the determination of extract in the coarse grind yield have only been accepted as tentative, and have been referred back to the Committee on Extract for further consideration. Your Associate Referee heads this committee.

It is proposed to have better methods for selecting the sample and for determination of the coarse grist yield than those now adopted. However, for the present, it is recommended[2] that the tentative methods be followed by the A.O.A.C.

No report on malt adjuncts was given by the associate referee.

REPORT ON CARBON DIOXIDE IN BEER

By P. P. GRAY (Wallerstein Laboratories, New York City), *Associate Referee*

No collaborative work was done during the year on this subject for the Association of Official Agricultural Chemists. However, continuing experience with the method in these laboratories gave no reason to doubt

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 73 (1938).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 73 (1938).

the accuracy and generally satisfactory character of the present tentative procedure.

In the previous collaborative work, three of the four reporting laboratories showed satisfactory agreement in results, so that the one laboratory reporting a deviating result may have encountered a freak bottle of beer.

In view of the accuracy indicated to be possible for the pressure method when the air is corrected for, as pointed out in a previous paper by Gray and Stone, *This Journal*, **19**, 162 (1936), it would be desirable to have available, at least as an alternative procedure for the use of those laboratories possessing the necessary apparatus, a method based on the determination of air and pressure and thereupon calculating the carbon dioxide content. A second paper from these laboratories on this subject[1] describes in detail the procedure to be followed in determining air and carbon dioxide. The Associate Referee recommends[2] that this pressure method, as described, be subjected to collaborative study in comparison with the present tentative chemical method. If the results of such collaborative tests are satisfactory, it would be desirable to adopt the new method as an alternative procedure.

REPORT ON WINES

By B. G. Hartmann (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

At the 1936 meeting it was recommended that a study be made of the saponification of esters occurring in wines by refluxing the depectinized sample with lead acetate solution. The saponification obviates the introduction of alkali, which is always undesirable in acid work. Moreover, lead saponification does not involve the introduction of an added step since in the procedures for organic acids the addition of lead acetate is prescribed.

After the sample has been prepared and depectinized in the customary manner, the resulting alcoholic solution is treated with the required quantity (A) of the lead acetate solution and refluxed for 30 minutes. After cooling, the material is washed into a centrifuge bottle and the lead salts, which now include the acids from the esters, are further treated as described in the procedure for the respective acid.

This work will be continued next year.

It is recommended[2] that study of the above subjects be continued.

No report on total sulfur and lead saponification was given by the associate referee as no collaborate work had been done.

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[1] *Ind. Eng. Chem. Anal. Ed.*, 10, 15 (1938).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 73 (1938).

REPORT ON VOLATILE ACIDS IN WINE

By M. A. JOSLYN (University of California, Berkeley, Calif.), *Associate Referee*

Much importance has been attached to the determination of the volatile acid content of wine since this factor is a good indication of its soundness and quality (1, 2, 3) though occasionally old wines are found to be of excellent flavor in spite of their relatively high volatile acid content (4). Of the many methods, *e.g.* (5), suggested for the determination of the volatile acidity the most widely used are those based on the steam distillation of 10 cc. of wine in some modification of the Hortvet apparatus (6), although the steam distillation of 50 cc. of wine suggested originally by Lindemann (7) is also used in the United States and abroad (8), and some modification of the Duclaux method (such as the Mathieu procedure) is occasionally used because of its speed.

Several investigators have called attention to the fact that the various procedures in common use give widely divergent results and that even the same distillation procedure may yield discordant results in the hands of several investigators (9). The failure to obtain concordant results by these methods has been ascribed to the incomplete removal of volatile acids (10) and to the entrainment of lactic acid (11) and production of acids by pyrolysis when excessive heating occurs, particularly in the presence of lime (10, 12). Many of the recent investigations have dealt with the errors caused by the presence of sulfur dioxide in wines, which have been shown to be of more importance than the entrainment of lactic acid and other fixed acids (13).

The steam distillation procedure has been subjected to several investigations (14) in this country and has been adopted as official. However, it was pointed out in the 1910 report that the character of the flame used and the rate of distillation affect the results, the smaller the flame the larger the increase in volume in the Sellier tube and the lower the volatile acidity obtained. However, the rate of distillation to be used has never been specified in any of the procedures given (15). In a recent publication Ionescu, Goal, and Popescu (16) stressed the importance of specifying the rate of distillation and pointed out that rate of distillation, concentration of acid in the solution, volume from which wine is distilled, and volume of distillate obtained all affect the accuracy of the determination. Ferré (2) also stressed the need of closely specifying the conditions of the determination, although in the method adopted by international covenant this is not done (17). Lesley (18) reported that the hydrogen ion concentration noticeably affects the rate of steam distillation of volatile acids. The discrepancies in the results obtained by the methods in current use for the volatile acid content of several wines by several commercial analysts and winery chemists indicate the need for somewhat closer specification for the methods.

TABLE 1.—*Results on volatile acidity obtained by several collaborators using different methods*

COLLABORATOR	DRY RED WINE	SWEET ANGELICA WINE	METHOD USED
	g./100 cc.	*g./100 cc.*	
C. H. McCharles, Calif. Dept. Public Health Lab.	0.096	0.024	10 cc. of wine steam distilled in a large Sellier tube sealed into liter Erlenmeyer flask, no trap, short condenser, distillation being continued until 10 cc. required but 1 drop 0.1 *N* NaOH (B.K.H. still No. 26304).
R. McGuire, Calif. Dept. Public Health Lab.	0.102	0.030	As above.
M. A. Amerine, Div. of Viticulture, Univ. of Calif.	0.139	0.045	A.O.A.C. Method II, tannin added, 100 cc. distillate, fading end point.
G. L. Marsh, Div. of Fruit Products, Univ. of Calif.	0.104	0.029	10 cc. steam distilled, 100 cc. distillate boiled 1 min., titrated hot. 1 liter round-bottomed flask, large Sellier tube, trap.
	0.110	0.030	10 cc. steam distilled, 80 cc. distillate caught, titrated cold.
	0.114	0.033	10 cc. steam distilled, 100 cc. distillate caught, titrated cold.
	0.098	0.027	10 cc. steam distilled, 80 cc. distillate caught, distillate boiled 1 min. and titrated hot.
	0 092	0.026	20 cc. steam distilled, 100 cc. distillate caught, distillate boiled 1 min. and titrated hot.
Commercial Laboratories			
A	0.115–0.117	0.039–0.042	50 cc. wine+20–30 cc. water, steam distilled until about 350 cc. distillate obtained.
B	0.103	0.0256	A.O.A.C., Method II, vertical condenser, 100 cc. distillate.
C	0.116	0.030	A.O.A.C. Method II, 60 cc. distillate.
D	0.107	0.041	10 cc. steam distilled in a modified micro-Kjeldahl still and 100 cc. steam distillate titrated.
E	0.104	0.032	A.O.A C. Method II, B.K.H. still.
Winery Laboratories			
A	0.099	—	A.O.A.C. Method II.
B	0.102	—	Filtered, 50 cc. distilled in large Sellier tube, 250 cc. distillate collected, distillate heated to boil, titrated hot; corrected for SO_2.
C	0.1140	0.0348	A.O.A.C. Method II, vertical condenser, 100 cc. distillate.

TABLE 1.—(*Continued*)

COLLABORATOR	DRY RED WINE	SWEET ANGELICA WINE	METHOD USED
	g./100 cc.	*g./100 cc.*	
D	0.108	0.030	A.O.A.C. Method II.
E	0.096	0.024	A.O.A.C. Method I, sample 50–200 cc.
F	0.0912	0.0264	A.O.A.C. Method II, B.K.H. still.
G	0.0888	0.0264	As above.
H	0.105	0.029	A.O.A.C. Method II, 100 cc. distillate, B.H.K. still.
I	0.104	0.026	Procedure as in Commercial Laboratory D.
J	0.103	0.027	A.O.A.C. Method II, 10 cc. steam distilled to 100 cc.
K	0.094	0.031	50 cc. steam distilled, 250 cc. distillate collected, brought to incipient boiling in beaker with glass rod, twirled to remove CO_2, titrated hot.
L	0.111	0.027	A.O.A.C. Method I. 50–500 cc.
M	0.11	0.039	A.O.A.C. Method II. 10 cc. B.K.H. still
N	0.105	0.030	A.O.A.C. Method II. 10–100 cc. B.K.H. still.
O	—	0.027	As above.

In the first test made by the associate referee in 1936, samples of Angelica wine having an extract content of 13.20 grams per 100 cc. and alcohol content of 19.47 per cent, and a dry red wine of 13.31 per cent alcohol content were sent to several collaborators for analysis. The results given in Table 1 show a rather wide divergence for both wines even in the hands of skilled analysts. The methods used, however, also varied. Several new modifications of the Hortvet type apparatus were in use. That used in this laboratory was the modification described by Lesley (18). The B. K. H. still was designed by McCharles and had the advantage of being all glass and compact. The modified micro-Kjeldahl still developed by J. Fessler, in which the rate of distillation was rapid enough to discharge the distillate hot, at about 60° C., and which was titrated hot in order to avoid error due to CO_2, has been further modified by Fessler and is, because of its convenience, finding favor in California. It is believed that some of the discrepancies found were due to difference in type of apparatus, for Fonzes-Diacon and Jaulmes (9), P. Jaulmes (19), and L. Ferré and P. Archinard (13) report that entrainment of lactic acid and other fixed acid may be prevented by the use of a small rectifying column or by means of a double trap, although Semichon and Flanzy (10) do not believe that this is so, since they have not found that the Fonzes-Diacon and Jaulmes apparatus prevents entrainment of lactic acid.

TABLE 2.—*Results on volatile acidity obtained by collaborators using Hortvet-Sellier apparatus and titrating hot and cold*

COLLABORATOR	CLARET		PORT	
	COLD	HOT	COLD	HOT
		g./100 cc.		
C. H. McCharles	0.093	0.093	0.060	0.051
M. A. Amerine	0.096	0.092	0.079	0.061
G. L. Marsh	0 102	0.094	0.085	0.057
Ray Dunn, Div. of Fruit Products, Univ. of Calif.	0.101	0.093	0.084	0.060
Commercial Laboratories				
A	0 096	0.104*	0.060	0.073*
B	0.096	0.091	0 060	0.048
C	0.097	0 097	0 079	0.074
D	0.096	0.096	0 066	0.063
Winery Laboratories				
A	0.091	0.092	0.070	0.072
B	0 123	0.105	0 096	0 078
C	0.0966	0 096	0.0780	0.0750
D	0.093	0.093	0.063	0.063
E	0 0864	0.0828	0.0564	0 0564
F	0 102	—	0.066	—
G	0 096	0 090	0 084	0 066
H	0.099	0 093	0.064	0.058
I	0.105	0.105	0.090	0.077
J	0 097	0.095	0 063	0 058
K	0.087	0.087	0.066	0.051
M	0 102	0.102	0 066	0.066
N	0 093	0.090	0 084	0.072
O	0 0915	0.090	0.069	0.057
P	0.094	0.096	0.080	0 070
Q	0 102	0 09	0.068-0 079	0.066-0 078
R	0.096	0.090	0.084	0.066

* 50-100 cc.

In the second trial a sample of California claret (14.43 per cent alcohol) and one of light port wine (17.75 per eent alcohol) were sent out with the following directions:

1. Pipet 10 cc. of wine into the inner Sellier tube of the particular volatile acid apparatus used. State kind and source of apparatus.
2. Steam distil 100 cc. into a 300 cc. Erlenmeyer flask marked by file at the point where it contains 100 cc.
3. Titrate cold. Report as cold titration.
4. Repeat procedure but bring distillate to boil and boil for 1 minute. Titrate hot and report as hot titration. Use wooden beaker tongs to avoid burning your hands.

It was believed that the hot titration, by obviating errors due to entrained carbon dioxide, would lead to more concordant results. To de-

termine the effect of heating on loss of acetic acid, G. L. Marsh of this laboratory made up a solution of acetic acid of approximately 0.100 gram per 100 cc., using previously boiled distilled water, and steam distilled it in the Lesley modification of the Hortvet type still (18); 80 cc. of distillate was collected in a 250 cc. Erlenmeyer flask, and the distillate was boiled for varying periods. The results obtained follow:

	cc. 0.1 N NaOH	*gram/100 cc.*
Before distillation		
Unheated	1.85	0.111
Boiled for 1 min.	1.75	0.105
After distillation		
(80 cc. distillate collected)		
Unheated	1.75	0.105
Heated to boiling	1.75	0.105
Boiled 30 sec.	1.73	0.104
Boiled 1 min.	1.66	0.100
Boiled 2 min.	1.60	0.096
Boiled 3 min.	1.50	0.090
Boiled 4 min.	1.40	0.084

Apparently boiling for 1 minute does result in only a moderate loss of acetic acid. Similar results were obtained when alcohol was added to the distillate to simulate a wine distillate.

The results of the analyses given in Table 2 indicate that more concordant results were obtained with the hot titration, particularly with the port wine.

For the third test samples of dry Sauterne type wine, 13.95 per cent alcohol and a low sulfur dioxide content, and a Sherry-Port blend containing 18.76 per cent alcohol were sent out. The wines used in the tests reported here were those used previously in collaborative examination of the methods for determining alcohol content (20). The results obtained are shown in Table 3. They are not particularly concordant, although the deviations are of the same order of magnitude as those found in previous collaborative trials (14). An analysis of the results given in Tables 1–3 is shown in Table 4, which is to be compared with the results of the last collaborative trial made by Hortvet in 1909 as given in Table 5. In order to obtain greater concordance it would be necessary to give some attention to the following points:

1. *Preparation of the wine.*—In order to prepare a wine for analysis, particularly to remove carbon dioxide gas, various methods have been proposed. The one commonly given directs that the sample be thoroughly shaken or poured for a short time from one vessel to another and filtered. Some analysts heat the sample in an open dish to incipient boiling, while others subject the wine to boiling under a reflux condenser, see Peynaud (13), H. C. Gore (14—1909), (15), and Astruc (21). Although in mature wines having a very low gaseous content such methods suffice, in new wines and in carbonated wines a more effective method may be needed. In

this connection Peynaud (13) reported that continuous agitation of wine under a vacuum for 1–2 minutes removed practically all the carbon dioxide, whereas bringing the wine to incipient boiling was not efficient, all of the carbon dioxide not being removed even after three such treatments, cf. H. C. Gore (5). The error due to carbon dioxide may be corrected for also by taking the distillate off warm or by bringing it to boil, a practice now followed by many winery analysts.

TABLE 3.—*Additional results on volatile acid obtained by collaborators*

COLLABORATOR	SAUTERNE			SHERRY-PORT	
	1*	2	3	1	2
			g./100 cc.		
B. G. Hartmann, Food & Drug Adm., Washington, D. C.	0.081–0 083	0 079–0.082	—	0.098–0.102	0 100–0.101
R. G. Love, Alcohol Tax Unit, San Francisco	0.077	0.075	—	0.099	0 096
M. A. Amerine	0.088	0.092	0 078	0 103	0 106
G. L. Marsh	0.078	0.073	0.072	0 102	0.091
Commercial Laboratories					
A	0.087	0.086	—	0 110	0.109
B	0.075	—	—	0.101	—
Winery Laboratories					
B	0 108	0 082	0.096	0 108	0 101
C	0.083	—	0 071	0.104	—
D	0.082	—	0 068	0.103	—
H	0.079	—	0.069	0 100	—
I	0.091	—	—	0 126	—
J	0.062	—	—	0 103	—
K	0.081	—	0 073	0 110	—
M	—	—	0.063	0 111	—
N	0 084	—	—	0 108	—
P	0.088	—	0.072	0 104	—
Q	0.090	0 060	0.080	0.108	0.080
S	0.108	—	—	0 108	—

* 1. 10 cc. of wine steam distilled in a Hortvet type still, 100 cc of distillate collected and titrated.
2. 50 cc. of wine steam distilled and about 350 cc of distillate collected and titrated
3. Results as in 1, but corrected for SO_2 as directed in *Methods of Analysis*, A O A C , 1935, p 167

Abroad some attention has been given recently to the matter of defecating and dealcoholizing the wine. Thus Semichon and Flanzy (10) have shown that wine treated with milk of lime and subsequently acidulated with tartaric acid gives higher results for volatile acidity than when not so treated, even when it is analyzed by the steam distillation procedure. This increase they attribute to the liberation of acids bound by the extractive materials. P. Jaulmes (12, 22), however, believes that the

extract has but little effect on the distillation of volatile acids from wine when the steam distillation method is used and that the increase in acidity found in treated wines is due to acids formed by the action of the lime on

TABLE 4.—*Volatile acid content of dry and sweet wine*

ANALYSTS	NUMBER REPORTING	AVERAGE	MAXIMUM	MINIMUM	NUMBER WITHIN ±0.1 CC.
			g./100 cc.		
		Dry Wine 1			
Commercial	8	0.103	0.116	0.096	5
Winery	14	0.106	0.139	0.089	7
All	22	0.105	0.139	0.089	13
		Dry Wine 2			
All, Cold	22	0.098	0.123	0.086	16
All, Hot	22	0.095	0.105	0.083	16
		Sweet Wine 1			
All	23	0.030	0.045	0.024	18
		Sweet Wine 2			
All, Cold	23	0.073	0.096	0.060	5
All, Hot	23	0.064	0.078	0.053	13

the sugars present in the hot solution. Peynaud (13) has called attention to the value of defecation with barium hydroxide for the purpose of removing sulfites as well as other substances.

TABLE 5.—*Collaborative results on tests of Hortvet distillation method for volatile acidity reported August 1909*

COLLABORATOR	WINE 1	WINE 2	WINE 3	WINE 4	WINE 5
			g./100 cc.		
J. R. Eoff	0.102	0.142	0.109	0.113	0.078
Azor Thurston	0.087	0.132	0.102	0.108	0.078
W. F. Sudro	0.108	0.135	0.120	0.126	0.071
H. C. Gore	0.098	0.129	0.111	0.116	0.084
Edmund Clark	0.096	0.135	0.099	—	—
Julius Hortvet	0.096	0.132	0.105	0.111	0.084
Number within ±0.1 cc	3	5	4	3	4

2. *Acidity of the wine.*—It is known that the hydrogen-ion concentration of the solution has an affect on the rate at which volatile acids distil over and it is likely that some suitable method for adjusting the *p*H of wine before distillation to some constant value may be desirable.

3. *Apparatus and rate of distillation.*—Several variations of the Hortvet

still are in use. These vary in size of Sellier tube, trap, and condenser, and in some instances the Sellier tube is immersed in boiling water and in others only in steam. The rate at which the wine is distilled, and the temperature of the distillate vary. It would be desirable to have the effect of these factors studied.

4. *SO_2 correction.*—The official method used at present (8) has been adopted abroad (17) although a variety of other procedures has been suggested (13). Its international adoption has been criticized recently by Marcille (23), but for all general purposes the method is satisfactory.

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(16) *Ann. inst. rech. Agronom. Roumanie*, **5** (4), 26 (1933);
Chem. Zentr., 2466, II, (1935).

(17) L. BENVEGNIN. *Mitt. Lebensm. Hyg.*, **25**, 222–3 (1934);
Ann. fals., **28**, 418–26 (1935).

(18) *Fruit Products J.*, **8** (11), 14–15, July (1929).

(19) *Ann. fals.*, **27**, 547–50 (1934).

(20) M. A. JOSLYN, G. L. MARSH, and J. FESSLER, *J. Assoc. Official Agr. Chem.*, **20**, 116–130 (1937).

(21) *Prog. agr. vit.*, **96**, 569–70 (1931).

(22) *Ann. fals.*, **28**, 540–5 (1935).

(23) *Bull. Direction Affaires Economiques Service l'Agr. Tunis*, **40**, No. 166, 381–387 (1936); *Ann. fals.*, **30**, 299–304 (1937).

REPORT ON SULFUR DIOXIDE IN BEERS AND WINES

By L. V. TAYLOR, JR. (American Can Co., Maywood, Ill.), *Associate Referee*

During the past year the Associate Referee studied four standard methods for the determination of sulfur dioxide in food products from the standpoint of accuracy and practicability when applied to alcoholic and carbonated beverages. The methods included in the investigation were, (1) Monier-Williams, (2) direct distillation, (3) steam distillation, and (4) Ripper. Of these methods, the Monier-Williams was the only one that was found to be universally applicable to these products under the conditions of the experiment. This work was summarized in a contributed paper published in *This Journal*, **20**, 610 (1937).

It is recommended[1] that the studies on sulfur dioxide methods be continued and in addition that collaborative work on the problem be conducted during the coming year.

REPORT ON ALCOHOL BY USE OF EBULLIOSCOPE

By PETER VALAER (Alcohol Tax Unit, Treasury Department, Washington, D. C.), *Associate Referee*

While most chemists determine quantitatively the amount of alcohol in alcoholic beverages and other alcohol-containing liquids by the methods now described in *Methods of Analysis, A. O. A. C.*, there is a much larger group that follows the far more popular method specifying an ebullioscope. In this latter group are 1200 wineries, 700 breweries, 340 rectifiers, and more than 300 distillers.

The procedure with the ebullioscope is comparatively simple, and it can be used on a fermented sample without distillation. However, if the sample is carefully distilled and made to original volume at the proper temperature, the probability of accuracy is enhanced.

The determination of alcohol by the use of the ebullioscope is based on the variation in the boiling point of mixtures of alcohol and water. While this principle is just as sound scientifically as the principles involved in the official densimetric and refractometric methods of analyses, the ebulliometric method is not generally regarded as an accurate procedure for alcohol determination. The use of a carefully standardized pycnometer, which determines accurately the specific gravity of a distillate, *Methods of Analysis, A. O. A. C.*, 1935, 148, is generally regarded as the most accurate method. However, like any other precise method, it is only as accurate as the person making the determination and is subject

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 74 (1938).

to errors due to the construction of the instrument, fluctuation of temperature, manner of weighing, evaporation, condensation, etc.

In their comprehensive report Joslyn and others, *This Journal*, **20**, 116 (1937), made the general statement that "with suitable precautions the alcohol content may be determined to ± 0.1 per cent by pycnometer and ± 0.2 per cent by ebullioscope. If the results do not fall into these ranges they are definitely in error."

During the past few years the Alcohol Tax Unit has examined and used the following named instruments and has approved their use in bonded wineries, breweries, rectifying houses, etc., as required by A. T. regulations: Salleron-Dujardin, Braun-Knecht-Heiman, Juerst (E & A), Tagliabue Twin, E. B. Torino, Levesque, Mallegand (petite & grand), Arnaldo-Sala, and Lefco.

Suggestions were made to dealers in some of these instruments regarding the protection of the thermometer from abnormal heating and the instrument from drafts under practical conditions of operation.

It may be stated in general that all of the ebullioscopes mentioned are based on the physical property, boiling point, and that they were found to be satisfactory for the purposes and within the limitations for which each instrument was designed.

It was found that the best results were obtained—

(a) When the use of the instrument was restricted to beer, dry wines, and similar substances in which the natural solid content did not exceed 5 grams per 100 cc.

(b) When the utmost care was taken in the dilution of natural sweet wines in order not to have the solid content over 5 grams per 100 cc. and to keep the alcohol content within the limitation of the instrument.

(c) When the wine, beer, cordial, distillery mash, or other alcohol-containing substance under examination was carefully distilled and the volume made up at the proper temperature.

(d) When all the conditions of the determination were normal, the directions supplied with each instrument were implicitly followed, and the calibration with water was made at frequent intervals.

(e) When the thermometer (the most important factor) was carefully standardized by the U. S. Bureau of Standards and any correction furnished by the maker was rigidly applied.

(f) When the flame was regulated carefully, the thermometer protected by a shield, and the whole instrument guarded well against drafts. (A uniform micro burner was found to be superior to the alcohol lamp.)

(g) When all dilutions necessary were carefully made and all parts of the apparatus and all glassware used were scrupulously clean.

In spite of its extended use, there seems to be no sentiment in favor of the adoption of the ebullioscope as an official method for the determination of alcohol. The manufacturers of the various instruments are con-

stantly striving for greater accuracy, but they have made little effort to have this instrument adopted as tentative or official. In court testimony it has been both accepted and rejected.

Any collaborative work should include all the ebullioscopes on the market, but this is difficult to manage because the distribution of taxable material is impeded more or less by legal technicalities, and very few chemists possess more than one ebullioscope. Wine and beer are sensitive to conditions and difficult to ship as samples.

There is also a grave question whether an error of ± 0.2 per cent alcohol would be permissible in an *A. O. A. C.* method. No referee would be likely to favor this procedure unless its operation were very carefully described and all the scales were based on the same kind of determination.

It is recommended[1] that no further effort be made by this Association to adopt the ebullioscope as an official or tentative method of alcohol determination.

No report on detection and adulteration of distilled spirits was given by the associate referee.

REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Although no collaborative work was done during the past year, the Associate Referee desires to report on the analysis of two cordials made in the laboratory according to a recipe evolved for the preparation of apricot and peach cordials and which seemed to be in keeping with recipes found in a number of books consulted.

A quantity of dried fruit (250 grams) was placed in a one gallon bottle, macerated with one liter of a mixture of equal parts of alcohol and water for ten days, and shaken several times each day. The pulp was then filtered off and extracted similarly a second time by maceration for seven days with 500 cc. of equal parts water and alcohol. After the pulp had been filtered off, the two extracts were mixed, 400 grams of sugar was dissolved therein, and the volume was completed to 1500 cc. with water.

Determinations of volatile acid, esters, and gamma-undecalactone were made after three months and again after nineteen months. The results are given in the table.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 20, 73 (1937).

	Apricot cordial		Peach cordial	
	June, 1936	October, 1937	June, 1936	October, 1937
	mg./liter	*mg./liter*	*mg./liter*	*mg./liter*
Volatile acid as acetic	137	38	101	32
Volatile esters as ethyl acetate	39.4	52.8	19.6	26.4
Gamma-undecalactone	None	None	None	None

The results indicate that no gamma-undecalactone is present naturally in either dried apricots or dried peaches.

During the coming year the Associate Referee expects to do additional collaborative work on the determination of esters and benzaldehyde in cordials and liqueurs, as recommended by Committee D in 1936.

The paper, entitled "Measurement of Beer Foam," presented by Morris A. Pozen, was published in *Brewery Age*, **5**, 43–46 (1937), under the title "Second Thoughts on Beer Foam."

The paper, entitled "Intensified Study of the Use of Refractometer as a Check in Beer Analyses," presented by Siebel and Kott, was published in *This Journal*, **21**, 121 (1938).

MONDAY—AFTERNOON SESSION

REPORT ON EGGS

By H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Unfortunately the press of other duties this year has interfered more than usual with the group of referees on eggs and egg products, so that only two reports by associate referees have been submitted. The Associate Referee on Decomposition recommends the adoption of a tentative rapid method for acidity of ether extract, which recommendation is approved. The General Referee also concurs in the recommendations for further study of methods and repeats the recommendations of last year for other contemplated work on eggs.

No report on unsaponifiable constituents and fat was given by the associate referee.

REPORT ON DECOMPOSITION IN EGGS

By JOSEPH CALLAWAY, JR. (U. S. Food and Drug Administration, New York), *Associate Referee*

Last year this Associate Referee reported on some collaborative work done on a method that would permit a relatively rapid determination of acidity of ether extract. Preliminary work indicated that this method gave results in reasonably close agreement with those obtained by the official method.

This year the Associate Referee again asked certain collaborators to secure samples of good and bad eggs and determine acidity of ether extract by the official method and by the rapid method proposed last year. In addition, request was made that lipoids be separated essentially as directed in the tentative method (*Methods of Analysis, A.O.A.C.*, 1935, 299, **10**) and that the lipoids so obtained be dissolved in benzene and titrated with sodium ethylate. It was thought that if the acidity of lipoids separated by the tentative method for lipoids was essentially the same as acidity of ether extract as determined by the official method, another method of separation such as proposed would not be necessary. Reports were received from H. I. Macomber of the Baltimore Station and from C. D. Schiffman of the Atlanta Station, U. S. Food and Drug Administration.

Macomber reports as follows:

On August 12, 1937, three eggs of good commercial grade were opened and allowed to stand about 24 hours at room temperature. On August 13, 1937, these liquid eggs had thickened and had a slightly strong odor. These eggs are lot No. II. On August 13, 1937, three other good eggs were opened and Numbered Lot I. Both lots were stored in the refrigerator until August 16, 1937. when they were mixed together and analysis by the three methods was started again. At this time lot No. II had a slightly sour odor and was very thick.

Because of the low acidity results obtained, the two combined lots were allowed to stand for 24 hours at room temperature and again analyzed on August 17, 1937. In spite of a strong, garlic odor and very thick consistency, the results were again low and the eggs were left at room temperature another 24 hours. On August 18, 1937, the eggs had a very strong putrid odor and the surface was slightly moldy.

When I first tried the proposed rapid method on lot No. II, which was very thick, I had difficulty in extraction. Because of the instructions to shake gently, I obtained only about 0.15 gram of extract. Subsequently, I found it necessary to shake vigorously until, on being allowed to settle briefly, the ether layer was colored yellow. No difficulty was encountered in separating the layers on centrifuging. This vigorous shaking was not necessary with the freshly broken eggs in lot No. I.

The proposed rapid method for the most part checks quite closely with the official method for acidity of ether extract. The acidity of the lipoids, however, checks the official method in only one out of the five tries, being nearly 0.5 cc. or more higher in the other four analyses.

DATE STARTED	LOT NO.	OFFICIAL A.O.A.C. METHOD			PROPOSED RAPID METHOD			TENTATIVE LIPOID METHOD		
				average			*average*			*average*
8/13/37	I	1.57	1.60	1.59	1.52	1.58	1.55	2.19	2.24	2.22
	II	1.49	1.41	1.45	1.33	1.44	1.39	1.53	1.49	1.51
8/16/37	I & II	1.59	1.62	1.61	1.37	1.29	1.33	2.31	2.34	2.33
8/17/37	I & II	1.10	1.22	1.16	1.18	1.27	1.23	1.60	1.78	1.69
8/18/37	I & II	2.54	2.62	2.58	2.87	2.83	2.85	2.86	3.03	2.95

Schiffman reports as follows:

Samples of good and bad shell eggs were obtained, and six samples were examined. The acidity of the egg fat was determined by the official and the proposed methods, also the acidity of the lipoids obtained by the official method was determined. The results shown in the next tabulation, are expressed as cc. of 0.05 *N* sodium ethylate.

The results by these collaborators indicate that the proposed rapid method gives results in close agreement with the official method. The method based on titration of separated lipoids according to the present tentative method for lipoids does not give as good results as the proposed new method.

It is recommended, therefore, that the rapid method be adopted as tentative and known as "Tentative Rapid Method for Acidity of Ether Extract." The method was published in *This Journal*, **21**, 85 (1938).

Sample No.	Per gram of fat — By proposed method	By official method	Per gram of lipoids by official method
1	16.75	18.63	21.52
2	2.30	2.47	3.08
3	2.56	2.50	2.58
4	2.17	2.19	2.60
5a	1.70	1.75	2.61
5b	1.64	1.78	2.63
6a	2.97	3.14	4.37
6b	3.14	3.14	4.23

5a and b are duplicates.
6a and b are duplicates.

In the light of the above results, it would seem that the proposed method could be depended on for close approximation of the official method.

Two collaborators were also requested to do some work on the method of Bandemer and Schaible for ammonia nitrogen. Last year Analyst Tubis reported to the Association that he obtained results by this method in fairly close agreement with those obtained by the present tentative method. In the method of Bandemer and Schaible ammonia is liberated from liquid egg by alkali and absorbed in standard acid. Both egg and acid are held in separate compartments in a covered dish in an incubator. In the present tentative method the egg material is suspended in alcohol and water and a current of air is blown through it, and then through a measured amount of standard acid. Absorption in a covered dish in an incubator requires much less manipulation and equipment. Only one report in comparative work was received this year. This was from Irwin S. Shupe, Food and Drug Administration, St Louis. Shupe reports as follows:

The rapid method for ammonia nitrogen in liquid eggs, *This Journal*, 20, 159 (1937), was compared with the tentative A.O.A.C. method, and the following results were obtained:

Sample	Tentative A.O.A.C. Method	Proposed Rapid Method
	NH_3 (*mg./100 g.*)	
A	8.0	11.0
B	3.5	6 3
C	3.5	7.9
D	1.5	3.1
	1.5	3.1

Absorption time of 5 hours was used for both methods. In the tentative A.O.A.C. method over 95% recovery was obtained in 3½ hours on 20 cc. of 0.02 *N* $(NH_4)_2SO_4$. In the proposed rapid method about 95% recovery was obtained at room temperature (25° C.) in 5 hours on 3 cc., 2 cc., and 1 cc. quantities of 0.02 *N* $(NH_4)_2SO_4$. Would not the lack of an inhibitor such as NaF in the proposed method permit a gradual decomposition to produce an apparently high ammonia figure?

Absorption dishes of the type described by Tubis were prepared and used, but all glass-divided, 2-compartment dishes would be more convenient and easier to clean.

Shupe obtained higher results by the new method. He suggests the possibility of continuing decomposition of egg material during the absorption. In the tentative aeration method sodium fluoride is added to inhibit bacterial decomposition. It would probably be advantageous to use this also in the absorption method. The Associate Referee will recommend that this be done next year.

The chemical methods for detection of decomposition in eggs that have been studied by the Association are based on detection of products produced by action of bacteria. Decomposition will usually have progressed to a considerable extent before such products of decomposition are present in quantities easily measured.

Certain changes take place in non-fertile eggs held at incubation temperatures where bacteria have not gained entrance. Studies of these changes are desirable. More fundamental research on the chemistry of eggs is necessary if progress is to be made in the detection of decomposition by chemical means.

It is recommended[1] that work on chemical methods of detection of decomposition in eggs be continued by the Association including further work on methods for ammonia nitrogen.

No report on glycerol, sugar, and added salt was given by the associate referee.

REPORT ON WATER-SOLUBLE NITROGEN AND CRUDE ALBUMIN NITROGEN IN DRIED EGGS

By F. J. McNall (U. S. Food and Drug Administration, Chicago, Ill), *Associate Referee*

Authentic samples of dried whites, yolks, and whole eggs, together with samples of the liquid eggs from which the dried eggs were made, were obtained at a commercial drying plant. The analyses of these samples made by the Associate Referee are given in the table. The results were calculated to the dry basis.

The dried whites obtained were very insoluble and could not be used for collaborative study, so a sample of egg albumin (impalpable powder soluble) was secured from a reputable manufacturer. The total nitrogen content of this sample, dry basis, was 14.37 per cent.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 69 (1938).

	Total nitrogen *per cent*	Water-soluble nitrogen *per cent*	Crude albumin nitrogen *per cent*
Liquid whites	14.41	13.44	11.56
Liquid whole eggs	7.97	4.59	3.23
Liquid yolks	5.48	1.20	.47
Dried whites	14.40		
Dried whole eggs	7.96		
Dried yolks	5.48		

This sample and the authentic dried whole eggs and yolks were submitted to six collaborators. Results were obtained from four collaborators. The tentative method for dried eggs, *Methods of Analysis, A.O.A.C.*, 1935, p. 298, was used.

Collaborative results calculated to dry basis

COLLABORATOR	DRIED WHITES		SPRAY-DRIED WHOLE EGGS		SPRAY-DRIED YOLKS	
	WATER-SOLUBLE NITROGEN	CRUDE ALBUMIN	WATER-SOLUBLE NITROGEN	CRUDE ALBUMIN	WATER-SOLUBLE NITROGEN	CRUDE ALBUMIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Rupert Hyatt	9.58	8.29	4.24	3.42	0.95	0 48
	9 58	8.38	4.14	3.35	0.92	0.43
K. L. Milstead	10.11	8 37	4.35	3.42	0 94	0 38
	10 11	8.50	4.34	3 42	0 94	0 38
J. N. Watkins	11.04	9 40	4.07	3 17	0 90	0.33
	11.00	9.37	4 07	3 13	0.90	0.34
V. E. Munsey	10 98*	9.38*	4 43	3 51	0 90	0 34
	11.27	9.68	4.35	3 44	0 88	0 31
F. J. McNall	11.39	9.82	4.35	3 44	0.88	0.34
	11.33	9.76	4.35	3.42	0 87	0 33
Average	10.64	9.10	4 27	3.37	0 91	0 37

* 25 cc. aliquot used instead of 50 cc.

COMMENTS BY COLLABORATORS

V. E. Munsey.—The filtrate as used under 7(a) for water-soluble nitrogen on white and whole eggs was not clear. Repeated filtration showed little, if any, improvement. If this condition is characteristic of these products, a modification of the present statement should be made in the procedure to conform with actual experience.

K. L. Milstead.—Difficulty was experienced in getting egg white to dissolve. Sample was shaken harder than method called for.

DISCUSSION

The collaborative results for water-soluble nitrogen and crude albumin in dried whole eggs and egg yolks are in fairly close agreement. The results for egg white are rather disappointing. Mitchell, in 1932, reported that vigorous shaking of the water extract of whites caused a precipitate,

the amount increasing with the time and vigor of the agitation. It is very likely that the low results reported by Hyatt and Milstead were due to prolonged and vigorous shaking. If the sample is broken up with a stirring rod and the water added slowly, very gentle shaking is all that is necessary to get the material disintegrated.

It is recommended[1] that the tentative method for the determination of water-soluble nitrogen and crude albumin nitrogen be adopted as official for dried eggs.

REPORT ON PRESERVATIVES

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Referee*

The principal studies on preservatives published during the past year seem to have been made by the English. Papers dealing with the determination of boric acid, sulfites, benzoic acid, and the compounds of *p*-hydroxybenzoic acid in foodstuffs have appeared in recent issues of the *Analyst.* In view of the reported increasing use of *p*-hydroxybenzoic compounds any article describing them has special interest.

Edwards, Nanji, and Hassan,[2] the investigators, have worked out a series of qualitative and quantitative procedures, some of which are applicable in the presence of benzoic acid, salicylic acid, and saccharin, and have applied these methods to milk, butter, cordials, sausages, and other foodstuffs. The results with many of these products are most promising.

EXPERIMENTAL

As recommended during the past year the Referee continued the study of the applicability to foodstuffs of the official method for the determination of saccharin in nonalcoholic beverages, *Methods of Analysis, A.O.A.C.*, 1935, 434. Two different brands of apple butter were obtained. To the first sample and a portion of the second, there was added enough of a solution of saccharin (acid form) to make 200 p.p.m. in the finished product. Saccharin was then determined by the official method and the new method on both these samples, and by the official method on the portion of the second sample to which no saccharin had been added. The results, including blanks obtained on the sodium carbonate-potassium carbonate fusion mixture appear on the next page.

COMMENTS

Official Method.—The fusion mixture was prepared from C. P. sodium carbonate and U.S.P. potassium carbonate. The latter salt was dried and powdered before being mixed, and the mixture was sifted rapidly and

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 69 (1938).
[2] *Analyst*, 62, 178–185 (1937).

Results on apple butter
(200 p.p.m. saccharin added)

COLLABORATOR	OFFICIAL METHOD		NEW METHOD
	BLANK $BaSO_4$	SACCHARIN	SACCHARIN
	mg.	*p.p.m.*	*p.p.m.*
	Sample A		
H. E. Chaney	5.5	235	229
	5.5	235	225
M. S. Oakley	3.3	228	225
	2.8	220	225
Referee	4.6	183	210
	3.8	175	215
	Sample B		
H. E. Chaney	5.0	175	
	4.9	180	195
M. S. Oakley	4 7	160	215
	5.2	165	225
	4.6		
	5.5		
Referee	5.2	180	205
	5.1	186	210
	Sample B (as purchased)		
Referee		39	
		31	

then stored in a tightly stoppered container. The variability of the blanks obtained indicates that the sulfate content of this mixture is too high and is unevenly distributed. This is a source of serious error as, under the conditions of the method, 1 mg. of barium sulfate is equivalent to 26 p.p.m. of saccharin.

When the method is run in duplicate excellent agreement of results is obtained. That these results depart from theoretical and vary among the analysts is explained by the uncertain and variable nature of the blank.

The two following minor departures from the official directions were tried and were found to facilitate the method.

(a) Ether is removed from the combined ether extracts by distillation until a volume of 20–25 cc. is reached; this is transferred to a 50 cc. beaker, reduced by evaporation to 5–10 cc., and then transferred to the platinum crucible. Although this procedure requires an additional container, it eliminates the necessity of attempting to transfer a small volume of concentrated solution from a half-liter flask to a 15 cc. crucible without loss.

(b) After addition of bromine water and hydrochloric acid the mixture is heated to boiling before being filtered. This removes gases, which clog the pores of the paper and interfere materially with filtration.

New Method.—The technic of the new method is quite simple, and the

results in this laboratory are rather promising. Certain modifications and observations follow:

(a) Recalculation of the factor converting ammonium chloride to the sodium salt of saccharin containing two molecules of water of hydration shows the value to be 1.317 rather than 1.39.

(b) After the second hydrolysis it was found necessary to filter the liquid before diluting to 50 cc.

(c) Tests employing 1 cc., 2.5 cc., and 5 cc. of Nessler's reagent indicate that 1 cc. is sufficient to produce maximum color and lessens the tendency of the liquid to become cloudy. Hence 1 cc. was used.

(d) In order to make a satisfactory comparison with color standards the range wherein the color change is most sensitive must be selected. Standard Methods of Water Analysis employs standards ranging from 0.001 mg. to 0.070 mg. ammonia nitrogen in a 50 cc. volume and these general limits were employed in this work. This necessitates using 2 cc. rather than 5 cc. of the dilute solution when 200 p.p.m. of saccharin is present.

(e) The majority of the comparisons were made with a Nessler's solution over a year old. When this supply was exhausted and fresh reagent prepared and used it was difficult to obtain a good match of color tint.

The Referee regrets that the pressure of work has prevented consideration of the other two recommendations. One of these, the Illing method for sodium benzoate, has been commented on by Edwards, *et al.* They report that it is "a very good test but unfortunately it is by no means specific for benzoic acid. Also, the accuracy of the method of determination based upon the test depends to a large extent on the stated time being allowed for nitration and strict adherence to other details."

It is recommended[1] that the method for the determination of saccharin in nonalcoholic beverages be further investigated and if found satisfactory submitted to collaborative study.

No report on benzoate of soda was given by the associate referee.

REPORT ON COLORING MATTERS IN FOODS

By C. F. Jablonski (U. S. Food and Drug Administration, New York), *Referee*

The recommendation of the committee last year requested the Referee to continue the collaborative investigation of the quantitative estimation of ponceau SX in presence of ponceau 3R.

With this end in view the Referee sent six sets of samples consisting of

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 74 (1938).

five subdivisions each to the collaborators with instructions to estimate the dyes by a submitted method, which is a modification of the one tried last year.

The samples in question were of the following composition (determined by $TiCl_3$ titration):

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—43.4 Ponceau 3R—42.7	86.1
2	Ponceau SX—65.0 Ponceau 3R—21.4	86.4
3	Ponceau SX—21.7 Ponceau 3R—64.1	85.8
4	Ponceau SX—86 7 Ponceau 3R— 0.0	86.7
5	Ponceau SX— 0.0 Ponceau 3R—85.2	85.2

The reports submitted by the collaborators follow:

O. L. Evenson, Food and Drug Administration, Washington, D. C.

SAMPLE NO.	*per ecnt*	TOTAL COLOR *per cent*
1	Ponceau SX—45.0 Ponceau 3R—40.4	85.4
2	Ponceau SX—66 0 Ponceau 3R—19.8	85.8
3	Ponceau SX—21.4 Ponceau 3R—64.1	85 5
4	Ponceau SX—86.7 Ponceau 3R— 0.0	86.7
5	Ponceau SX— 0.0 Ponceau 3R—84.9	84.9

Mrs. A. P. Bradshaw, Bureau of Chemistry and Soils, Washington, D. C.

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—49.0 Ponceau 3R—36.7	85.7
2	Ponceau SX—70.5 Ponceau 3R—15.8	86.3
3	Ponceau SX—24.9 Ponceau 3R—62.2	87.1
4	Ponceau SX—87.5 Ponceau 3R— 0.0	87.5
5	Ponceau SX— 0.0 Ponceau 3R—84.4	84.4

S. S. Forrest, Food and Drug Administration, Washington, D. C.

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—46.2 Ponceau 3R—39.5	85.7
2	Ponceau SX—67.6 Ponceau 3R—18.8	86.4
3	Ponceau SX—19.8 Ponceau 3R—66.5	86.3
4	Ponceau SX—85.2 Ponceau 3R— 0 6	85 8
5	Ponceau SX— 0.0 Ponceau 3R—85.7	85.7

L. Koch, H. Kohnstamm and Co., New York.

SAMPLE NO	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—47.2 Ponceau 3R—37.6	84.8
2	Ponceau SX—71.1 Ponceau 3R—15 7	86.8
3	Ponceau SX—22.6 Ponceau 3R—63.8	86.4
4	Ponceau SX—89 6 Ponceau 3R— 0.0	89.6
5	Ponceau SX— 0.0 Ponceau 3R—84.4	84.4

J. Morris, H. Kohnstamm and Co., New York.

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—47.0 Ponceau 3R—37.3	84.3
2	Ponceau SX—72.6 Ponceau 3R—14.2	86.8
3	Ponceau SX—23.4 Ponceau 3R—61.6	85.0
4	Ponceau SX—88.0 Ponceau 3R— 0.0	88.0
5	Ponceau SX— 0.0 Ponceau 3R—84.4	84.4

J. L. Hogan, Food and Administration, New York.

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—47.1 Ponceau 3R—38.8	85.9
2	Ponceau SX—68.7 Ponceau 3R—16.9	85.6
3	Ponceau SX—25.1 Ponceau 3R—60.4	85.5
4	Ponceau SX—86.2 Ponceau 3R— 0.0	86.2
5	Ponceau SX— 0.0 Ponceau 3R—84.3	84.3

The following comments and criticism were offered by the collaborators:

O. L. Evenson.—In regard to Sample 4, there is a possibility that some ponceau 3R may be present. An average value was taken in calculating the amount of ponceau SX present and this indicated the absence of the ponceau 3R.

S. S. Forrest.—In general, the method seems to be satisfactory, check results being obtained within 0.5 per cent. However, in the case of Sample 4, where the ponceau 3R must be less than 1.0 per cent, it is impossible to say that it is present or absent; 0.1 cc. difference in the mean titration would allow about 0.5 per cent ponceau 3R. Mean values are given for Sample 4, showing 0.62 per cent ponceau 3R. It would seem then that if ponceau 3R is present in very small amounts, the slight difference in titration values would preclude the possibility of saying with certainty that ponceau 3R is present or absent.

A. P. Bradshaw (Mrs.).—In the case of Sample 4, the consumption of $TiCL_3$ by ponceau SX after treatment appears slightly greater than the total consumption before treatment, but this difference is so slight that we feel it is due only to experimental error, and the sample was calculated as ponceau SX.

L. Koch and J. Morris.—The results obtained for Sample 4 gave a negative value, which would indicate that something was wrong. Nothing could be found that would invalidate the method of analysis. The procedure gave consistent results, but it was obvious that a slight error in titration was magnified five times when the calculation was completed. It was further noted that the absorption of the dye by the ferric hydroxide was of such a magnitude and nature that it could not be washed out, and also prevented the analysis of mixtures containing 2.5 per cent or less of ponceau SX by completely removing the dye from the solution, giving a yellow liquid on filtration.

J. L. Hogan.—Generally speaking, no difficulties were encountered in obtaining sharp end points in the titrations. Check determinations also agreed fairly well. However, in Sample 4, using the factor correction, I obtained a small negative quantity. But since the original titration was less than the corrected titration, ponceau 3R was considered to be absent.

DISCUSSIONS

When it is considered that 0.1 cc. of 0.1 N titanium trichloride corresponds to 0.6 per cent of dye, the results shown are rather encouraging. It must further be mentioned that in some instances the collaborators obtained results closely approaching theory.

For Sample 1 the extremes reported for ponceau SX were 2.8 per cent and for ponceau 3R, 3.7 per cent. For Sample 2 the extremes reported were 6.6 per cent for ponceau SX and 5.6 per cent for ponceau 3R. For Sample 3 the extremes reported were 5.3 per cent for ponceau SX and 6.1 per cent for ponceau 3R. In Sample 4 all but one of the collaborators reported the presence of ponceau SX only. A number of the collaborators noted a small discrepancy between the original titration and the titration after oxidation with the factor correction. The extremes reported in Sample 4 as ponceau SX were 4.4 per cent. In Sample 5 all collaborators agreed that the dye consisted only of ponceau 3R. The extremes reported for Sample 5 were 1.4 per cent.

In examining the results reported by the collaborators, it is evident that in these color mixtures the figures for ponceau SX are invariably higher (with one exception), while the figures reported for the corresponding ponceau 3R are lower than the theory. These errors may be due entirely to an error in the graph submitted by the Referee.

As a matter of fact this was noted by one of the collaborators (L. Koch), who after considerable investigational work, suggested a correction of the graph and further stated that the addition of ferrous sulfate is not necessary to destroy the excess of hydrogen peroxide, and, furthermore, that the precipitated ferric hydroxide occludes some of the ponceau SX.

A second report submitted by Koch for the same samples follows:

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—44.0 Ponceau 3R—40.9	84.9
2	Ponceau SX—67.0 Ponceau 3R—19.9	86.9
3	Ponceau SX—21.6 Ponceau 3R—64.8	86.4
4	Ponceau SX—90.0 Ponceau 3R— 0.0	90.0
5	Ponceau SX— 0.0 Ponceau 3R—84.4	84.4

Another collaborator (J. L. Hogan) repeated his analysis on the same samples, using a slight modification of the method. The results reported follow:

SAMPLE NO.	*per cent*	TOTAL COLOR *per cent*
1	Ponceau SX—43.2 Ponceau 3R—42.8	86.0
2	Ponceau SX—64.5 Ponceau 3R—21.2	85.7
3	Ponceau SX—21.6 Ponceau 3R—64.0	85.6
4	Ponceau SX—86.2 Ponceau 3R— 0.0	86.2
5	Ponceau SX— 0.0 Ponceau 3R—84.3	84.3

These last reports are a decided improvement over the first results reported by the same collaborators, notably the last report, which is close to theory.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be continued in estimating ponceau SX and ponceau 3R in mixtures.

(2) That investigational work be continued for the quantitative estimation of sunset yellow FCF in presence of tartrazine.

(3) That investigational work be undertaken to separate and estimate quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

The paper, entitled "Volatile Oil in Marjoram," presented by J. F. Clevenger, was published in *This Journal*, **21**, 109 (1938).

REPORT ON METALS IN FOOD

By H. J. Wichmann (U. S. Food and Drug Administration, Washington, D.C.), *Referee*

ARSENIC

The results of collaborative experiments intended to complete the sample preparation part of the arsenic project did not terminate as anticipated. One collaborator, using the xanthate extraction and molybdenum blue determination, obtained results quite different from the other collaborators who used the Gutzeit method. No definite reason for

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **21**, 74 (1938).

this difference can be given at present and it will be necessary to repeat the collaborative work.

The work of the Associate Referee on Arsenic on the mercury content of impregnated Gutzeit strips is interesting and suggestive. If there is a range of about 8 per cent in the mercury content of strips from the same sheet, or even from end to end of the same strip, it raises the question whether this variation is responsible in part for the admitted error of 5–10 per cent of modern official Gutzeit determinations. It has always been the belief of the Referee that the errors of the Gutzeit method are due largely to the small differences in the rate of arsine evolution, but the associate referee's mercury results suggest that another unexpected factor must be considered. It may be necessary to look for other fibers more uniform than paper in absorption capacity, or treat the impregnated strips in some manner that would equalize the mercury concentration.

It is problematical whether the many and ever increasing critical conditions of the Gutzeit method can ever be so rigidly controlled as to bring the error of its results into line with other modern micro methods. If greater accuracy can be gained it will probably be at a sacrifice of simplicity and convenience. The Referee therefore believes it advisable to promote efforts designed to develop other procedures to follow after the arsine evolution or other methods of isolation. The associate referee has suggested three possible ways of attack: (1) Iodine titration of arsine according to a modification of the original Smith[1], and Taber, *This Journal*, **13**, 417 1930; **14**, 436 (1931), mercuric chloride procedures, and possibly suitable for 5–500 gamma quantities; (2) molybdenum blue formation according to Zinzadze after absorption of arsine in mercuric chloride; (3) color formation by the action of arsine on solutions of salts of easily reduced metals. The Referee believes that the older process of arsenic isolation by distillation as $AsCl_3$ or $AsBr_3$ and the very modern idea of extraction of arsenic as the xanthate complex[2] should be considered in connection with final determination, particularly the molybdenum blue method. The associate referee points to the formation of the red gold sol as a color that can be read in a photometer and that forms a straight line standardization curve. Silver also forms a sol with arsine, more silver per unit of arsenic being reduced because of its monovalency, but it has greater photochemical activity, which might become troublesome in determinations. The Referee wishes to point out that if these gold or silver sols can be stabilized by some colloid so that the particles are small enough, and do not agglomerate in a reasonable time, a simple, sensitive, and rapid method for arsenic could be developed because these sols should

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[1] U. S. Dept. Agr. Bur. Chem. Circ. 102 (1912).
[2] Abstract of paper by Klein and Vorhes presented at 1936 Meeting of this Association. Details will be published later in *This Journal*.

lend themselves to rapid readings with a photometer or a photoelectric colorimeter.

ANTIMONY

The Referee wishes to discuss antimony in connection with the subject of arsenic. It is his understanding that a Gutzeit method for antimony that is less sensitive and accurate than the corresponding arsenic method is about to be published. (See p. 314.) This may be useful as an interim method, but if the sensitivity and accuracy are as represented, the Referee does not believe that the long and involved history of the Gutzeit method for arsenic in this Association should be repeated. He suggests that if the iodine titration or the gold or silver sol methods for arsenic are developed to a satisfactory degree, there seems to be no theoretical obstacle to the development of corresponding methods for antimony.

Other phases of determination of both antimony and arsenic that the Referee believes important are their separation and determination when they exist together in micro quantities. Up to this time antimony has been generally ignored. In the few instances where both arsenic and antimony were suspected, the arsenic was usually separated by arsenious chloride distillation with temperature control, or magnesium ammonium arsenate precipitation, and the antimony discarded, or the arsenic was determined without separation by the molybdenum blue method, which does not produce blue color formation with antimony. As a consequence there remains no worth-while background on micro antimony determinations.

The literature mentions methods of arsine and stibine separation that may bear investigation at this time. Metallic iron and acids are said to evolve arsine, but not stibine. Zinc or aluminum in caustic alkali produces arsine but reduces antimony compounds only to the metal. These methods of hydride production might not be suitable for Gutzeit operation, but they probably have little influence on the three methods of determination suggested by the associate referee. Arsine and stibine production, concurrently or separately, is an oxidation-reduction phenomenon, and an examination of the problem of arsenic and antimony determinations in the light of electrode reduction potentials might yield handsome dividends.

The problems of antimony and arsenic are so closely allied that the Referee recommends that they be combined under one associate refereeship. He also appeals for help for the new associate referee, who is expected to farm out different parts of the project to collaborators rather than do all the development work himself. The Referee believes that splendid methods for the determination of both arsenic and antimony may be developed along newer lines. The prospect is of absorbing interest. Will those interested and willing to take part in this development inform the writer or the Associate Referee on Antimony and Arsenic.

COPPER

The Associate Referee on Copper reports briefly that he has determined the absorption curve for the copper-carbamate complex and has experimented with two filters, one in the green and one in the far blue. Both of these filters appear to be suitable. What remains to be done is the determination of the absorption curve of bismuth, the only serious interference, and the selection of a solvent that involves completeness of extraction. The associate referee believes that he can solve these remaining problems shortly and then be able to submit a method to collaborators based on the work of the previous associate referee and a photometric system of color measurement. Under such promising conditions the Referee is pleased to recommend that work be continued.

FLUORINE

The collaborative work of the Associate Referee on Fluorine, as exemplified by application of the determination to phosphates, indicates all too clearly that there is still something to be desired in the method when it is a question of small quantities. The variations are too great in relation to the quantity determined. The Willard-Winter distillation must be studied further relative to the reasons for incomplete recoveries of fluorine or the effect of substances that distil over with it. The total effect becomes noticeable when the amount of fluorine to be distilled becomes smaller. It seems that the associate referee's job is that of unscrambling small compensations of errors. Recovery experiments may show good results and still be affected by compensations. Sample preparation problems also require attention if the demand for a better method for small quantities of fluorine in all sorts of materials is to be met. Methods of determination after isolation may perhaps be improved, but at the present time the bottle neck of the fluorine determination from the standpoint of the time and difficulties involved is the isolation. In his 1935 report the Referee expressed the opinion that Willard and Winter had solved the problem of fluorine isolation and that only sample preparation and final determination remained for study. That opinion must be revised. There is much to be done on all three fronts, and the only recommendation that can be made under the circumstances is one for continued efforts on the fluorine problem.

LEAD

The Associate Referee on Lead conducted collaborative work with the lead methods intended for spray residues. One of his samples was treated with a collection of interfering substances that would hardly be expected on commercial apples in either such quantity or variety. These interferences have been mentioned previously as causing difficulties with determinations, but not all of them are ever present at the same time. The associate referee therefore gave these methods a severe test. The only

difficulty encountered by the collaborators was with the silica, which caused the associate referee to suggest the addition of a sentence to the present tentative method that would avoid future trouble when excessive silica might be present.

The data submitted by the associate referee and other information scattered through much official correspondence justify the recommendation for official adoption (first action) of the rapid methods restricted to apples and pears described in *Methods of Analysis*, Sections **30, 31, 32,** and **33,*** 391. The other lead methods are not ready for such action. The partial digestion methods of sample preparation, a checkup on the ashing temperature of carbohydrate products, sample preparation in the case of oils, fats, or fatty products, and the elimination of interferences need more individual or collaborative attention. These objectives will keep the next associate referee quite busy.

The Referee has watched the lead sulfide method, particularly with reference to the analysis of spray residue on fresh fruits, with the object of observing its vitality in competition with the newer methods. The empirical nature of turbidimetric methods in general seems to operate against its chances of survival. However, this method, especially the photoelectric modification, is still in use for spray residue purposes in the eastern part of the United States, which indicates that it has merit. This is probably due to the small particle size of the fresh precipitate and the speed with which measurement can be made by the newer photoelectric methods. The Referee does not believe that the sulfide method will again seriously challenge the dithizone method for accuracy or sensitivity for low amounts, unless possibly for restricted purposes, as in the spray residue field. If the advocates of the sulfide method for lead in spray residues desire to continue its use and to give it tentative status in competition with the newer methods, the Referee believes the Association should place the initiative and burden of proof upon them.

MERCURY

Since the extraction of mercury by dithizone from solutions containing organic matter may be uncertain and erratic, the complete oxidation of organic matter without loss of mercury is a task that involves some difficulties as well as much of the analyst's time and attention. The associate referee therefore tried first to concentrate the mercury and separate it from the greater part of the organic matter. The precipitation with finely divided zinc of mercury contaminated with a small amount of readily oxidizable organic matter appears feasible.

The associate referee tested Fischer and Leopoldi's[1] "mixed color"

* See appendix to the report of the Associate Referee on Lead in reference to the effect of lime sulfur in the wash solution (practised in December, 1937) on the colorimetric dithizone and excess sugar from soft or decayed apples or improper stripping of apples on the direct electrolytic method (Sec. 33). This will necessitate slight changes in the wording of Sections 30 and 33 next year.

[1] *Z. anal. Chem.*, 103, 241 (1935).

duplication dithizone method intended by the authors for the 0–10 gamma range and a "two-color" photometric method similar to Clifford and Wichmann's lead method on the isolated mercury, *This Journal*, **19**, 130 (1936). His results on the Fischer method in a 1–100 gamma range showed an accuracy of about 1 gamma, which may be considered a corroboration of Fischer's results. The accuracy of the Fischer method and that of the associate referee's titrametric method, now tentative, are about on a par, and the Referee believes, therefore, that it is useless to spend more time on it unless it can be shown that it has advantages in convenience and accuracy not now apparent. The associate referee might consider it further from the standpoint of its usefulness in the low 0–10 gamma range.

In an attempt to secure greater sensitivity and accuracy, the associate referee modified the photometric lead method to fit mercury conditions. The filter selected measures the absorption of the excess dithizone rather than that of the mercury complex. The method therefore becomes an indirect one and depends on the stability of the dithizone reagent. The associate referee has not yet demonstrated the superiority of either system, and therefore much remains to be done in the way of development and collaboration.

A micro method for mercury has been very useful on a number of occasions this past year. The Associate Referee on Arsenic used it to determine the variation of mercury impregnation of Gutzeit arsenic strips. It has also been used in the study of the penetration of mercury compounds into wood and other agricultural products. Use of mercury in the spray residue field has so far been rather limited; if it should increase, micro methods will be available when the need arises. The Referee expects that increasing use of the dithizone mercury methods may be made in the analysis of pharmaceuticals.

SELENIUM

The work of the Associate Referee on Selenium this year shows (1) that the available simple open-system sample preparations apparently do not result in appreciable selenium losses; and (2) that a starch-iodine titration can be substituted for last year's electrometric titration with no loss of accuracy and a considerable gain in convenience and simplicity. He shows (3) that the titration of selenium with thiosulfate and iodine solution yields results with a small, fairly constant error of 0.2–3.0 gamma, according to the range of the selenium and the concentration of reagents. This causes a high percentage error in the lower members of a given range. Dilution of reagents, use of micro burets, titration in small volumes, and the proper fitting of concentrations to amounts to be determined, all lower these errors, but 5 gamma of selenium seems to be the minimum that can be determined without undesirable increases of error. In the

Referee's opinion an error of 5–10 per cent of the amount present should not be exceeded in good microanalytical work. Where the amount of selenium to be determined is so small that this percentage of error is likely to be exceeded, even with all the proper precautions mentioned above, another method is needed. Sufficiently satisfactory results should be obtainable by the thiosulfate method to fill, perhaps, 90 per cent of all requirements. The other 10 per cent, comprising especially the 1–10 gamma range, must be left for future development, probably along colorimetric lines.

If the associate referee can first determine definitely whether it is safe to prepare samples in open containers in all cases, or if condensation of distillates is a necessary precaution, the stage should be ready for extensive collaborative work on samples that might, for practical purposes, be set within the 10–1000 gamma range. Any additional time available to the associate referee should be devoted to the investigation of methods especially suited for 0–10 gamma quantities.

ZINC

Difficulties of interference, since dithizone is not a specific reagent, seem to be particularly evident in the case of zinc. Fischer[1] and recently Sandell[2] have recommended the use of thiosulfate or potassium iodide at a *p*H of 4.0–4.5 to preferentially fix interfering metals into complexes and leave only the zinc to combine with dithizone. The optimum *p*H for the extraction of zinc is above 4.5, but these reagents decrease in their complex-forming ability as the *p*H increases. Their use, therefore, is subject to some criticism. The associate referee suggests sodium diethyldithiocarbamate, the reagent used in copper determinations, for the preferential complex formation. This reagent produces colored compounds with copper and bismuth, and colorless ones with other metals, many of them soluble in non-aqueous solvents according to the report of Associate Referee Coulson last year. The Associate Referee on Zinc believes that, in conjunction with dithizone, the carbamate reagent will remove the interference of some metals in solutions too acid for the extraction of zinc and form preferential complexes more stable than the dithizone complexes in weakly ammoniacal solutions with others, and leave the zinc free to combine with dithizone without any interference.

The associate referee does not present definite data in support of this theory, but does offer a number of recovery experiments with spinach ash. These experiments seem promising, but the Referee believes that the underlying theory should be studied further before extensive collaborative studies are initiated. A number of zinc methods have been presented recently, and the Association must exercise some discretion in order to utilize its limited time to the best advantage.

[1] *Z. anal. Chem.*, **107**, 241 (1937).
[2] *Ind. Eng. Chem. Anal. Ed.*, **29**, 464 (1937).

The associate referee extracts excess dithizone with 0.02 *M* ammonia before comparing the solutions. This is the so-called "one-color" system. It depends for success upon choosing the *p*H at which the solution of the dithizonate can be washed with sufficient care so that no metal is partially transferred to the aqueous phase, or compensating any small error in washing by making the same error with the standards. The Referee believes that photometric reading at the proper wave length or with a color filter will obviate the necessity for removing excess dithizone and give more accurate determinations as well. He recommends this also to the attention of the next associate referee.

Perlman, *This Journal*, **20**, 627 (1937), reports good results in determining zinc in maple products of the order of 8–422 p.p.m. He extracts the zinc with some other metals with dithizone and then finally determines the zinc iodometrically with ferricyanide and potassium iodide. This reaction is said to be specific for zinc. Since 1 cc. of 0.001 *N* thiosulfate is equivalent to 100 gamma of zinc, the method probably lacks sensitivity in the lower ranges (0–25 gamma), and larger samples must be prepared for the determination. The prospects with respect to the determination of zinc seem to point towards the possibility of dividing it into three broad ranges with some overlapping, viz., 0–100 gamma by colorimetric dithizone methods, 50–2000 gamma by dithizone extraction and iodometric titration, and above 2 mg. by the present tentative method. It is recommended that the associate referee pay special attention to the two lower ranges.

RECOMMENDATIONS

It is recommended[1]—

(1) That the study of methods of sample preparation of refractory products containing arsenic be continued for another year.

(2) That the iodine titration, gold or silver sol colorimetric, and the molybdenum blue methods for the determination of arsenic be further studied as possible substitutes for the Gutzeit method.

(3) That the study of methods for the determination of antimony be combined with those for arsenic under one associate refereeship, because of the close relationship of these elements, and that their separation when existing together in micro quantities in organic or biological materials be studied.

(4) That study of methods for the determination of micro quantities of copper be continued.

(5) That the study of fluorine methods be continued.

(6) That the colorimetric dithizone and the electrolytic methods intended for the rapid determination of lead on apples and pears in Secs. **30, 31, 32,** and **33** of Chapter 29 of *Methods of Analysis, A.O.A.C.*, be

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **21**, 71 (1938).

made official (first action). (New information received since the meeting will require some rewording of Secs. **30** and **33**.)

(7) That work be continued on the general methods for lead.

(8) That the problems of isolating mercury from solutions containing organic matter and its photometric determination be further studied, and that collaboration be initiated as soon as the first two objectives have been accomplished.

(9) That an extensive collaborative study of the thiosulfate method for selenium be begun and that a search be made for a method especially suitable for the 0–10 gamma range.

(10) That studies intended to develop workable methods for the determination of zinc be continued.

REPORT ON ARSENIC

By C. C. Cassil (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

SAMPLE PREPARATION

Collaborative experiments designed to finish that part of the arsenic project dealing with sample preparation did not turn out as anticipated. Composite samples of shrimp and tobacco were sent to three field stations for arsenic analysis by the perchloric acid digestion and dry ashing procedures recommended by the Associate Referee in the 1936 report, *This Journal*, **20**, 171 (1937). The results obtained by these collaborators and the Associate Referee are given in Table 1.

Table 1.—*Results obtained with shrimp and tobacco*

Analyst	Shrimp			Tobacco		
	Perchloric acid treatment	H_2SO_4-HNO_3 digestion	Dry ashing	Perchloric acid treatment	H_2SO_4-HNO_3 digestion	Dry ashing
	mmg. As_2O_3 in 10 gram sample					
C. W. Murray	200	—	175	300	—	280
J. E. Fahey	205	172	—	315	230	—
C. C. Cassil	215	93	210	305	—	325
H. Bois*	186	180	33	370	365	371

* The final determination was made by the xanthate-molybdenum blue method.

H. Bois, using a xanthate extraction and molybdenum blue method of determination, did not obtain results comparable to those obtained by the others using the Gutzeit method. Reasons for this discrepancy can not be given without further investigation.

C. W. Murray reports low results on both the shrimp and tobacco

when they were analyzed by the dry ashing procedure. This loss is probably due to insufficient ashing, as he did not obtain a white ash. He also reports that the ash mixture had a tendency to spatter when placed in the muffle and some of the sample may have been lost in this manner.

J. E. Fahey had no muffle at his disposal and therefore was unable to carry out the dry ashing procedure. The differences between perchloric digestion and sulfuric-nitric acid digestion reported by Fahey are not so great as those found by the Associate Referee. This is probably due to a longer and more complete digestion than that usually employed by the latter.

It would appear that the perchloric acid treatment is effective in destroying the refractory substances present in such products as shrimp and tobacco, but the evidence is not complete owing to one set of results. Therefore it will be necessary to do more collaborative work in sample preparation during the coming year. Minor changes in the dry ashing procedure will be made and the collaborative work repeated.

ANOTHER VARIABLE IN THE GUTZEIT METHOD

Factors governing the evolution of arsine in the Gutzeit method have been considered the cause of the errors encountered in this procedure. Mercury determinations made on Gutzeit strips impregnated with different concentrations of mercuric bromide and for different periods suggest that variation in the mercury content of the individual strips may be another important factor. Considerable variation has been shown from strip to strip and from end to end on the same strip when treated at the same time under identical conditions. The results of the mercury determinations are given in Tables 2 and 3. Saturation does not occur until the strips have been treated for 2 hours, and after 17 hours there is a range of 8.3

TABLE 2.—*$HgBr_2$ variation in individual strips, 3.5 per cent $HgBr_2$ in 95 per cent alcohol*

TIME OF IMPREGNATION	STRIP NO.	WEIGHT OF STRIP	$HgBr_2$ ABSORBED
hours		*mg.*	*mg.*
½	1	32.2	1.86
	2	30.3	1.24
1	3	32.1	1.91
	4	30.7	1.93
2	5	34.3	2.73
	6	31.4	2.33
17	7	31.1	2.67
	8	31.7	2.76
	9	33.5	2.60
	10	33.4	2.74
	11	33.7	2.69
	12	30.5	2.53

TABLE 3.—*$HgBr_2$ variation from end to end on the same strip when impregnated with 1.5 per cent $HgBr_2$ for 3 hours*

STRIP NO.	END OF STRIP	$HgBr_2$ ABSORBED
		mg.
1	Lower	0.277
	Upper	0.270
	Total	0.547
2	Lower	0.243
	Upper	0.262
	Total	0.505
3	Lower	0.229
	Upper	0.256
	Total	0.485
4	—	0.575
5	—	0.570
6	—	0.564

per cent and a standard deviation of 3 per cent in the amount of mercury absorbed. No way to correct this error is apparent. Mercury absorption is a function that determines the degree of uniformity and porosity of the individual strips, and the possibility of finding a more nearly uniform fiber to substitute for the strip should be considered.

OTHER METHODS OF DETERMINING ARSENIC

Owing to the simplicity and rapidity of the Gutzeit method, it is favored for certain uses over all other methods previously suggested, but it also has well recognized limitations. Variables, such as type of zinc, impregnation of strips, etc., which can not be rigidly controlled, make the official Gutzeit method empirical, as it has an inherent error of 5 to 10 per cent. The need for more accurate methods for determining small quantities of arsenic has opened this field of investigation. Three methods were studied this year, and these are described below in the order of their apparent merit.

(1) *Iodine Titration.*—The most promising micro method is an iodine titration, the principle of which was first given by C. R. Smith.[1] Winterfeld, Dörle, and Rauch[2] also described a method of this type in 1935, but their procedure was longer and more involved than that described below. The exact details of the procedure have not yet been worked out, but the method used is described briefly as follows:

The Gutzeit apparatus is modified by replacing the strip tube with a bent tube, the lower end of which is inserted into a test tube holding an absorbing solution. The evolution bottle contains 10 cc. of HCl, 5 cc. of 15% KI, 0.5 cc. of 40% $SnCl_2$, 4 grams of granulated Zn, and the sample (total volume not to exceed 50 cc.). The receiving test tube is so constructed that 1 cc. of solution (16 mg. $HgCl_2$+0.5 mg. gum arabic) gives a column of liquid 5 cm. high when the delivery tube is inserted.

[1] U. S. Dept. Agr., Bur. Chem. Circ. 102 (1912).
[2] *Arch. Pharm.*, 273, 457 (1935).

As soon as the Zn is added and the evolution bottle assembled (at room temperature), the evolution bottle is placed in a water bath kept at 80° C., and the evolution allowed to proceed for 5 minutes. At the end of this time evolution will be complete and approximately 1000 cc. of H_2 will be evolved, which is sufficient to reduce and evolve up to 500 mg. of As_2O_3.

The receiving tube is then disconnected and 2 cc. of 10% $Na_2HPO_4 \cdot 10\ H_2O$, 2 cc. of 15% KI, and an excess of 0.0025 *N* I are added. The excess I is back-titrated with a standard solution of As_2O_3, and the amount of arsenic in the sample is calculated on the basis of the I titer divided by 4. (The titer is divided by 4 because the iodine is oxidizing not only As_2O_3 to As_2O_5 but also HgCl to $HgCl_2$.) The HgCl is formed according to the equation, $2AsH_3 + 12HgCl_2 + 3H_2O \rightarrow As_2O_3 + 12HgCl + 12HCl$. In effect, this titation is the equivalent of the I oxidizing the AsH_3 to As_2O_5.

Results obtained by this procedure are given in Table 4. They are representative of the recoveries to date, but when certain difficulties are straightened out, it is believed that the error should not be more than 1 per cent.

TABLE 4.—*Arsenic in oxidized state before evolution*

As_2O_3 ADDED	0.0025 *N* IODINE	As_2O_3 RECOVERED	
mmg.	*cc.*	*mmg.*	*per cent*
0	0.02	—	—
5	0.17	4.9	98.4
10	0.29	8.9	89.0
15	0.48	15 0	100.0
20	0.62	19.6	98.2
25	0.78	24.9	99.5
30	0.92	29.4	98.1
40	1.21	39.0	97.6
50	1.51	48.8	97.6
75	2 27	73 6	97.0
100	2.97	97.0	97.0
130	3.85	125.5	96.6
160	4.69	153.0	95.8
200	5.97	197.0	98.5
300	9 02	297.0	99.0
400	11.98	396.0	99.0

(2) *Molybdenum Blue Method.*—A new method for isolating arsenic preparatory to the development of the molybdenum blue color was investigated. The regular procedure, which has been described by Zinzadze[1] and others, requires an arsenic trichloride distillation, oxidation with nitric acid, evaporation to dryness, and resolution of the arsenic oxide in hydrochloric acid. Although not tedious, this procedure requires about 2 hours to prepare a sample after the original digestion is complete, whereas the new procedure requires only about 10 minutes.

The new isolation step is carried out exactly as described under (1)

[1] *Ind. Eng. Chem. Anal. Ed.*, 7, 227 (1935).

Iodine Titration. When the evolution is complete, the contents of the receiving tube are transferred to a 50 cc. beaker and an excess of bromine water is added to oxidize the calomel and arsenic to the divalent and pentavalent states, respectively. The excess bromine is discharged from the solution either by boiling or by adding a few drops of phenol solution.

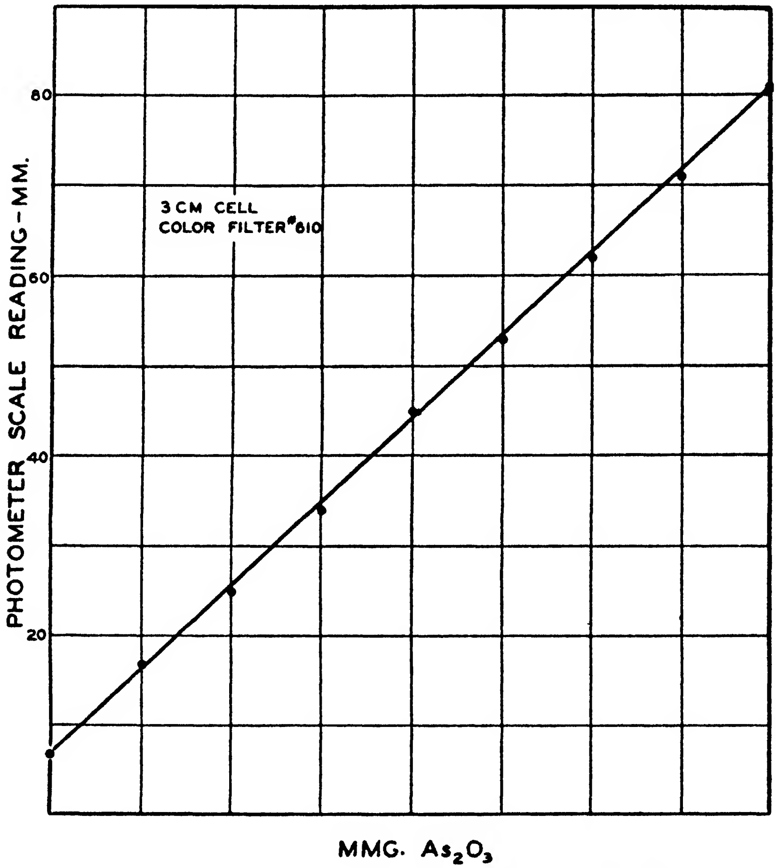

FIG. 1.—RESULTS OBTAINED BY THE SUGGESTED MOLYBDENUM BLUE COLORIMETRIC PROCEDURE FOR ARSENIC

This completes the sample preparation, and the color is developed by adding Zinzadze's molybdenum reagent and boiling the solution for 5 minutes. The complex arsenic-molybdenum compound is stable for 4 days if the proper acidity is maintained. With quantities of arsenic from 0 to 80 mg., the final volume is most suitable at 25 cc. and the amount of molybdenum reagent to be used is 0.25 cc. A neutral wedge photometer has been used for the estimation of color. Mercuric chloride does not interfere with the production of the blue color, and it adds no color to

the blank determination. The color is exactly the same whether the arsenic is carried through the isolation step or developed directly.

Results obtained in this manner are presented graphically in Fig. 1. The depth of color is directly proportional to the quantity of arsenic present. This method of arsine isolation followed by color development according to Zinzadze is worthy of further investigation.

Colloidal Gold Sol.—Arsine reduces gold trichloride, and in the presence of a protective colloid, such as gum arabic, the red gold sol produced is stable for about one hour. After that time the color intensity increases, perhaps because of a further reduction of the excess ionic gold by the action of the protective colloid, or perhaps because the first reaction proceeds rapidly but changes the arsine only to arsenous oxide and a secondary reaction of arsenous oxide to arsenic pentoxide proceeds slowly. Several protective colloids were tried, but none seemed more effective than the gum arabic. Again the depth of color can be measured in the neutral wedge photometer, and the color intensity is directly proportional to the quantity of arsine. This method also is worthy of further consideration.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative studies on sample preparation be continued.

(2) That the three methods of determination presented in this report be further studied.

REPORT ON COPPER

By D. L. Drabkin (University of Pennsylvania, School of Medicine, Philadelphia, Pa.), *Associate Referee*

The spectrophotometric absorption curves of amyl alcohol and carbon tetrachloride extracts of the copper-carbamate compound were determined. Unfortunately, these curves indicate maximum absorption in the deep blue or near ultra-violet region, where the visual acuity is none too good, but it is necessary to tolerate this condition. Two filters were studied in a preliminary way—one with transmission in the blue (Wratten No. 75), another in the green region Although theoretically the blue filter should be superior, thus far more concordant results have been obtained with the green filter, which transmits a region along a moderately sloping part of the absorption curve of the copper compound. This filter is the Wratten No. 62, Hg Green, mounted in B glass. It transmits maximally at wave length 530 mu, the total transmission being 3.5 per cent of the incident light. With this filter satisfactory results in accord with Beer's law are obtained with an ordinary colorimeter, when artificial

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 70 (1938).

light strained through a ground glass (not through "daylight" glass, which should be avoided) is used as a source. With photoelectric methods of matching intensities, both filters studied give excellent results.

The factors of completeness of extraction of pigment by solvent, and amount of solvent recovered under various conditions have not received sufficient attention. Also, it is believed that other solvents should be tried upon the copper-carbamate complex. Recently 2-methoxyethanol has been found to greatly intensify the ferric sulfocyanate color. It may work similarly with the copper-carbamate, and seems worth trying.

The points as to the light absorption characteristics of bismuth, with a view to the possibility of removing this interfering substance, and the control of *p*H, as suggested by the Referee on Metals in Foods, must be looked into. When more extensive work has been done along these lines, it is believed that a tentative method, which will prove acceptable, may be proposed to the Association for adoption.

REPORT ON ZINC*

By R. A. CAUGHEY, E. B. HOLLAND, and W. S. RITCHIE, (Department of Chemistry, Massachusetts State College, Amherst, Mass.), *Associate Referee*

During the past year the Department of Experiment Station Chemistry, acting in the capacity of Associate Referee, devoted considerable time to methods for the determination of zinc in plant products. The literature was reviewed at some length, and extensive preliminary work was conducted along lines suggested by various writers. Some of the gravimetric methods were reported to be practicable for paints, etc., but they did not meet the requirements of accuracy and speed essential for the work intended where only minute amounts of zinc are present.

As colorimetric methods have been used regularly in this laboratory for the determination of iron, copper, manganese, and phosphorus, and as zinc was known to produce colored bodies (complexes) with several organic compounds, a similar method for zinc seemed to be more promising, provided the intensity of the color produced was proportional to the content and the interference of other reacting elements could be eliminated.

Zinc reacts with resorcinol to produce a blue color in an ammoniacal solution and a red in hydrochloric acid, as shown by Cerdan and Puente.[1] The red is stable, and both colors are extractable. On repeated trials the zinc was found to act as a catalyst, as reported by Mohler and Widmer,[2] but not proportionally.

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* Contribution No 286, Massachusetts Agricultural Experiment Station.
[1] *Anales soc. españ. fis. quim*, 11, 98–108 (1913).
[2] *Mitt. Lebensm. Hyg.*, 22, 130–3 (1931).

As diphenylthiocarbazone (dithizone) was known to form highly colored compounds with various metals (15 or more), particularly lead, an attempt was made to use this reagent for the determination of zinc. Dithizone is extremely sensitive to metals and produces a green salt (complex) in a hydrochloric acid solution and red in an ammoniacal solution, both extractable with immiscible organic solvents. This permits a four-fold partition into aqueous and solvent fractions from acid and alkaline media. Furthermore, the metals vary in their ability to form stable salts in an acid medium, $p\mathrm{H}_2$ to $p\mathrm{H}_7$, but readily react in an ammoniacal solution, $p\mathrm{H}_7$ to $p\mathrm{H}_{10}$. The red compound is generally used for colorimetric measurement and varies from violet to red or orange, depending on the metal present and the amount.

Previous experience with the carbamate method for copper and with resorcinol for zinc provided some insight as to the necessary procedure. In the extraction of an ammoniacal ash solution with an organic solvent, the use of ammonium citrate was deemed necessary as a buffer to prevent the precipitation of iron, aluminum, calcium, and phosphoric acid, as in the carbamate process for copper. Carbon tetrachloride was considered preferable to chloroform or isoamyl alcohol as the solvent. The occurrence of zinc in nearly all the reagents required rigorous purification or the application of an unwieldy blank.

Eventually the extraction of a pure zinc dithizone salt was secured; it followed nearly a complete recovery between 5 and 25 gamma, but in the presence of copper and lead the results were less satisfactory and the color of the carbon tetrachloride extract indicated the presence of other metals. To obviate this difficulty carbamate reagent and an excess of dithizone were added to the original hydrochloric acid solution of the ash, after which they were shaken out with carbon tetrachloride and discarded. This procedure removed at least a portion of the interfering metals.

To the aqueous layer were added ammonium citrate, ammonium hydroxide, the carbamate reagent, and an excess of dithizone. The mixture was reshaken with carbon tetrachloride, and the aqueous portion was removed by suction. The carbon tetrachloride layer was washed twice with 10 ml. portions of 0.02 *M* ammonium hydroxide. The solvent containing the colored zinc salt was then drawn off into a weighing bottle, and the color was compared in a Duboscq colorimeter against a standard zinc solution treated in exactly the same manner. The procedure outlined gave a satisfactory separation of zinc. Solutions prepared by wet combustion (sulfuric and nitric acids) did not prove satisfactory, but they warrant further trial as a means of saving the time and labor required for ashing. In practice, 0.2 or 0.5 gram of material is used, depending on the ash content and the zinc thought to be present.

The method follows:

REAGENTS

(a) *Standard zinc solution.*—To 0.2000 gram of Zn, foil or granulated, add 5 ml. of HCl (1+1) and 2000 ml. of water, q.s. at 25° C.; dissolve the Zn in the acid and make to volume. 1 ml. contains 0.0001 gram of Zn.

(b) *Dilute zinc solution.*—To 200 ml. of standard Zn solution (preferably 40 ml. to a liter and use 5 ml. in the test to insure greater accuracy in measurement) add 2000 ml. of water), q.s. at 25° C. 1 ml. contains 0.00001 gram of Zn.

(c) *Ammonium citrate solution.*—Dissolve 200 grams of citric acid or 220 grams of NH_4 citrate in water, add NH_4OH until alkaline to litmus, and make to 2000 ml. Transfer 500 ml. to a separatory funnel, add an excess of dithizone, and shake out with 25 ml. of CCl_4. Discard the solvent layer and filter the aqueous portion.

(d) *Ammonium hydroxide.*—2 *N*. Distil NH_4OH at about 70° C. into redistilled cold water and dilute to the required strength.

(e) *Ammonium hydroxide.*—0.02 *N*. To 20 ml. of 2 *N* NH_4OH add 2000 ml. of water, q.s. at 25° C.

(f) *Hydrochloric acid.*—2 *N*. Distil HCl into redistilled cold water by means of a separatory funnel dripping below the surface of H_2SO_4 and dilute to the required strength.

(g) *Diphenylthiocarbazone (dithizone).*—0.10%. Transfer 0.1 gram of dithizone to a separatory funnel with 100 ml. of 0.02 *N* NH_4OH and shake out with 5 ml. of CCl_4. Discard the solvent layer and use the aqueous portion. Prepare a fresh solution daily.

(h) *Sodium diethyl dithiocarbamate (carbamate).*—0.25%. Dissolve 1.25 grams of carbamate in redistilled water and make to 500 ml.

(i) *Carbon tetrachloride.*—Distil over anhydrous Na_2SO_4.

(j) Redistilled water.—All water must be redistilled from glass to eliminate metals.

PROCEDURE

Transfer 0.20 or 0.50 gram of finely ground (1 mm.) air dry material, containing about 0.00001 gram (10 p.p.m.) of Zn to a Pt ash dish and incinerate in an electric muffle below visible redness. Take up the ash with 3–5 ml. of water and 1 ml. of the HCl, transfer to a 60 ml. pear-shaped separatory funnel, and make to a volume of about 25 ml. Add 1 ml. of the carbamate solution, 5 ml. of the CCl_4, and an excess of the dithizone solution (indicated by the green color of the CCl_4), shake out, and discard the solvent layer.

To the aqueous portion add 2 ml. of the NH_4 citrate, 1 ml. of the carbamate solution, 1.5 ml. of the 2 *N* NH_4OH, 10 ml. of the CCl_4, and an excess of dithizone solution (indicated by the orange color of the aqueous layer). Shake out and remove the aqueous layer by suction, wash twice with 10 ml. portions of the 0.02 *N* NH_4OH in the same manner and draw off the CCl_4 containing the colored zinc salt into a glass-stoppered weighing bottle.

Compare the color in a Duboscq colorimeter, using micro cups and a green color filter against 1 ml. of the dilute Zn solution treated in exactly the same manner after the addition of 1 ml. of the HCl. The color of the Zn-dithizone complex is fairly stable, but it should be compared as soon as possible for the best results.

$$\% = \frac{SR}{R_1} \times 500 \qquad \text{(or 200 for 0.5 gram)}$$

$$= \frac{0.005R}{R_1} \qquad \frac{0.002\,R}{R_1}$$

$$\text{p.p.m.} = \frac{50R}{R_1} \qquad \frac{20R}{R_1}$$

About 2 gamma of zinc is found in the blank after careful purification of all reagents, which would seem to indicate contamination by the glass containers. Therefore, $[(S+B)]/R_1R$ should be substituted in the calculation and finally the blank deducted.

The few results appended show that a satisfactory separation is obtainable.

Recovery of zinc from spinach ash

	R	R_1	ZINC FOUND	MINUS BLANK	ZINC PRESENT
			gamma	*gamma*	*gamma*
Blank	5	28.0	2.17	—	—
Blank	5	28.0	2.17	—	—
Standard 10 gamma	20	20.0	12.17	10 00	—
Spinach ash	20	23 4	10 40	8 23	8.23
Spinach ash	20	22.7	10.72	8.55	8.55
Spinach ash	20	22.8	10 68	8 51	8.51
Spinach ash	20	22 9	10.63	8.46	8.46
Ash+2.50 gamma zinc	20	18.4	13 23	11.06	8.56
Ash+2.50 gamma zinc	20	18 6	13.09	10.92	8.42
Ash+5.00 gamma zinc	20	15 2	16.01	13.84	8.84
Ash+5.00 gamma zinc	20	15 8	15 41	13 24	8 24

Separation of zinc from lead

	R	R_1
Zinc 20 gamma	5	5.0
Zinc 20 gamma	5	5 2
Zinc 20 gamma+lead 20 g.	5	5.3
Zinc 20 gamma+lead 20 g.	5	5 2

ADDITIONAL REFERENCES

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Hibbard, P. L. A Dithizone Method for Measurement of Small Amounts of Zinc. *Ind. Eng. Chem. Anal. Ed.*, **9**, 127–131 (1937).

Cerdan, Angel del Campoy and J. de la Puente. 1913.

It is recommended[1] that the Association subject the method presented in this report to additional investigation and invite the collaboration of other laboratories.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 71 (1938)

REPORT ON FLUORINE IN FOODS

By DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

For collaborative work two samples were distributed, No. 1 being dicalcium phosphate and No. 2, a water solution containing 15 p.p.m. of fluorine, 50 p.p.m. of phosphates, and 1000 p.p.m. of sulfates.

The methods suggested were:

I The peroxidized titanium method;

IIA The thorium nitrate titration applied to an aliquot of the distillate;

IIB The same method applied to the evaporated distillate; and

III Microtitration, as suggested by Armstrong.[1]

The following collaborators reported: W. S. Allen, General Chemical Co.; W. D. Armstrong, University of Minnesota; R. W. Bridges, Aluminum Company of America; J. N. Carothers, Monsanto Chemical Co.; J. R. Davies, General Foods Corp.; W. K. Enos, Virginia Carolina Chemical Corp.; J. B. Fullerton, The Upjohn Co.; M. Given, Eastman Gelatine Corp.; V. L. Harnack, United Chemical and Organic Products; Arthur D. Holmes, The E. L. Patch Co.; F. Visser't Hooft, Lucidol Corp.; Simon Klosky, American Agricultural Chemical Co.; H. V. Moss, Monsanto Chemical Co.; W. C. Motz, Virginia Chemical Corp.; K. B. Peterson, North Dakota Regulatory Dept.; G. D. Richards, Jaques Manufacturing Co.; W. E. Stokes, Standard Brands, Inc.; O. I. Struve, Eastern States Coop. Milling Corp.; F. O. Taylor, Parke, Davis & Co.; A. R. Wreath, Victor Chemical Works; and the following chemists in the U. S. Food and Drug Administration: Samuel Alfend, Jonas Carol, J. T. Field, E. M. Hoshall, J. A. Kime, J. H. Loughrey, Paul A. Mills, Manuel Tubis, Harley G. Underwood, Jos. H. Watkins, and the Associate Referee.

The results, listed in the order in which they were received, are summarized in Tables 1 and 2.

These results were treated as follows:

For each sample and method, average and standard deviations were calculated, as well as the range (average $\pm$ standard deviation) within which two-thirds of the results might be expected to fall. Table 3 gives these data.

For Sample 1, Table 3 shows that no matter which one of the four methods is used, a limited number of analyses fall within the range 10.48–11.64. Similarly for Sample 2, the range 12.05–16.19 is found common to all four methods.

Since a comparison of all reported results fails to indicate any one of the methods as definitely superior in *all* cases, the conclusion seems justi-

[1] *Ind. Eng. Chem. Anal. Ed.*, 8, 384 (1936).

TABLE 1.—*Results reported on Sample 1*

ANALYST	METHOD I		METHOD IIA		METHOD IIB		METHOD III		REMARKS
	AV.	VARIATION	AV.	VARIATION	AV.	VARIATION	AV.	VARIATION	
1	10.8	10.2–11.3	11.9	10.7–13.3	14.5		25.0	20.5–32.0	
2	10.0	10.0–10.0	13.0	13.0–13.0	11.5	11.0–12.0	9.3	9 0– 9.5	
3	9.1	9.0– 9.2							
4	10.5	10.0–11.0	10.3	10.0–11 0	11.0	11 0–11.0			
5	11.0		12.0	10.5–14.0	10.6				
6	7.0	7.0– 7.0	9.0	8 5– 9.8			23 8	19.3–28.3	
7	11.0	10.0–12.0	12.5				13 7		
8	11.0		8.0				10.0		
9	8.9	7.5–10.0	13.7	10 0–16.0					
10	9.9	9.7–10.0							
11	10.0	10 0–10.0	7.0						
12	7.0								
13	12.5	12.5–12.5	12.3	12.3–12 3					
14	8.0	6.0–10.7	10.9	9.6–13.1					
15			11.2	10.9–11 5					
16	13.0	12.0–14.0	12.0	12.0–13.0					Own method: 17.0
17			13.0						
18	11.0	9.0–12.0							Own method: 11.0
19	8.0	8.0– 8.0	12.0	10.0–14.0					Own method: 12.0
20			17.5	15.0–20.0	21.9	19.6–23.5			Own method: 14.2
21	9.1	5.0–13.3	13 0	11.2–15.1					Own method: 11.5
22	12.1	10.3–13.8	16.8	4.3–36.0					Own methods: 12.6 and 13.3
23	11.6	11.1–12.5	12.2	11.1–13.4	12.4	11.7–13.2	10 7	10.5–11.0	
24							20.6	20.4–20.8	
25	8.0	8.0– 8.0	25.0	22.0–28.0					
26	11.2	11.0–11.3	11.7	11.2–12.1			12.2	11.7–12.6	
27			14.9	14.0–16.0	15.1	14.8–15.2	16.0		
28							18.5		
29									Own method: 5.2
30	8.5	8.5– 8.5	14.1	14.0–14 1	10.3				
31	9.4	9.0–10.4							
32	10.9	5.8–15.7	11.4	5.2–17.5			15.5		Own method: 12.4

TABLE 2.—*Results reported on Sample 2*

ANALYST	METHOD I		METHOD IIA		METHOD IIB		METHOD III		REMARKS
	AV.	VARIATION	AV.	VARIATION	AV.	VARIATION	AV.	VARIATION	
1	15.3	13.3–18.2	14.1	13.8–14.4			18.0	14.1–19.8	
2	13.0	13.0–13.0	16.0	16.0–16.0			13.0	13.0–13.0	
3	14.6	14.5–14.8							
4	15.0	14.0–16.0	16.5	16.5–16.5	14.0				
5	16.0		16.8	16.0–17.5	14.2				
6	11.8	10.8–12.9	12.2	11.8–12.5			24.5	23.5–25.5	
7	13.0	12.5–13.5	13.5				13.7		
8	9.0		9.0				10.0		
9	5.2	4.5– 6.3	14.3	10.0–18.0	20.0	19.0–21.0			
10	9.4	9.2– 9.5							
11	12.4	12.0–12.8							
12	7.5								
13	12.5	12.5–12.5	13.5	13.3–13.8	13.5				
14	18.9	15.3–26.0	13.5						
15			13.5	13.5–13.5					
16	16.0	16.0–16.0	13.1	13.0–13.3					Own method: 12.8
17			12.7						
18	14.0	13.0–15.0							Own method: 14.0
19	12.0	10.0–13.0	11.0	9.0–13.0					Own method: 13.0
20			18.6	8.9–26.3	13.8				Own method: 15.2
21	12.0	6.7–16.7	11.7	10.0–14.6					Own method: 15.8
22	22.3	19.5–25.0	12.8	7.6–17.9					Own methods: 19.3 and 20.4
23	13.6	13.3–14.6	13.9	12.9–14.9	14.3		13.1	12.7–13.8	
24							19.7	19.0–20.4	
25	11.0	9.0–13.0	18.5	17.0–19.0					
26	13.8	13.5–14.0	14.5	14.1–14.7			15.7	14.8–15.9	
27			15.4	15.0–15.6	15.6	15.2–16.0	16.0		
28							14.1		Own method: 9.9
29									
30	7.3	7.0– 7.5	12.8	12.7–13.0	9.8				
31	10.3	10.0–11.0							
32	13.3	11.5–15.5	12.3	11.3–13.2			18.9		Own method: 13.4

fied that the results falling within the range common to all four methods might be called good.

All the methods show too great a variation between the results of different analysts. A part of this can no doubt be laid to lack of experience, since in reading the comments made by several analysts the Associate Referee noticed that the analysts expressing decided preference for one particular method usually (not always) found more consistent results by the method of their choice.

The fact that Sample 2 contained 15 p.p.m. of fluorine added as NaF with purity of 99.8 per cent opens a field of speculation. Thus it appears

TABLE 3.—*Summarized data*

SAMPLE NO.	METHOD USED	AV.	STD. DEVIATION	AV ± STD. DEVIATION
		p.p.m.		*p.p.m.*
1	I	9.98	1 66	8.32–11 64
1	IIA	12 73	3 51	9.22–16.24
1	IIB	13.41	3 86	9 55–17 27
1	III	15 94	5.46	10 48–21.40
2	I	12.77	3.65	9.12–16 42
2	IIA	13.92	2.27	11.65–16.19
2	IIB	14 40	2 81	11.59–17.21
2	III	16.06	4.01	12.05–20.07

that for these rather small quantities of fluorine, the methods tend to give slightly low results (exception is the Armstrong method). Since Methods I, IIA, and IIB have the double distillation and the evaporation in common, it would be natural to search for possible errors in this part of the procedure.

Two collaborators, Motz and Moss, reported finding a blank on the distillation. The Associate Referee has made a limited study of these questions of distillation blank and incomplete recovery, as well as of the suggestion made by Motz that the substitution of Vitreosil for glass beads would eliminate the blank. While the data are yet too meager to permit final conclusions, the indications are—

(1) That there exists a small blank due to the equipment used.

(2) That this blank is materially reduced but not wholly eliminated when Vitreosil is used instead of glass beads.

(3) That the recovery in the distillation is incomplete, about 0.02 mg. of fluorine apparently being retained.

(4) That this compensation of error in the Willard-Winter distillation ordinarily seems to be of no practical importance. When the amount of fluorine in the distillation flask is decreased, however, as is often the case when small quantities of fluorine occurring naturally in many materials

are to be determined, it may become necessary to consider this possible source of error.

It is recommended[1] that the work on fluorine in foods be continued.

REPORT ON LEAD

By P. A. Clifford (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

SPRAY RESIDUE METHODS

By far the largest proportion of regulatory work on lead concerns its determination as spray residue. For this reason it was considered advisable to stress spray residue methods first. The tentative procedures proposed last year, viz., the colorimetric dithizone and electrolytic procedures, have been in extensive use during the past five years, and their limitations and advantages have become well recognized.

The colorimetric dithizone procedure makes use of the solvent or "stripping" method of sample preparation, which lends itself well to routine work on account of its speed. Removal of spray residue lead by this process may not be complete but should approximate 95 per cent or better. In any event the error due to incomplete stripping probably lies below the error due to sample variation.

During the time the method has been in use it has been claimed that various interferences cause errors in results—silicate and phosphate by precipitating out with the alkaline earth metals under the conditions of the color development, causing low results due to lead occlusion; zinc by reacting with dithizone to cause high results; and sulfur by precipitating lead sulfide, which may be undissolved and lost upon filtration of the acidified strip solution.

The electrolytic method of lead determination on apples and pears "ties in" with the colorimetric method, as the analysis is made upon a larger volume of the same filtrate. The electrolysis can be made directly if manganese and chlorides are absent, but if either or both are present a preliminary isolation of lead by means of a dithizone extraction is prescribed.

For the collaborative work outlined here two solutions consisting of the acidified and filtered strip solution of unsprayed apples were sent to each collaborator; 1400 grams of apples was used per each 550 cc. of solution. Solution A contained no interferences but Solution B represented, per each 550 cc., 100 mg. *each* of Zn added as the acetate, Mn added as the arsenate and sulfate; S as dry lime sulfur; As_2O_3 as manganese arsenate ($MnHAsO_4$); P_2O_5 as sodium phosphate; Ca as the chloride and lime

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 70 (1938).

sulfur; Mg as the chloride; and SiO_2 as analyzed commercial sodium silicate. An amount of lead representing 0.0180 grain per pound was added to Solution A in the form of pure lead nitrate, and an amount equivalent to 0.0140 grain per pound was added to Solution B. The lead, and interferences in the case of Solution B, were added to the alkaline oleate strip solution *before* acidification and filtration. Nitric acid was used for both rinse and acidification.

TABLE 1.—*Collaborative results expressed as grain per pound*

COLLABORATOR	SOLUTION A (0.018)		SOLUTION B (0.014)	
	COLORIMETRIC	ELECTROLYTIC	COLORIMETRIC	ELECTROLYTIC
1	0.0178	0.0168	0 0138	0.0118
2	0.0178		0.0140	
3	0.0168	0.0174	0.0066*	0 0131
			0.016	
4	0.0180	0.0177	0.0142	0 0131
5	0.0186	0.0186	0.0143	0.0125
6	0.0185	0 0183	0.0144	0 0145
7	0.0186	0.0184	0.0073*	0 0145
			0.0173	
8	0.0186	0.0160	0.0128	0.0114
9		0 0176		0.0136
10		0.0180		0 0141
11	0.0181	0.0179	0.0139	0.0136
12	0.016		0.011*	
			0.016	
Average	0.01788	0.01767	0.01467	0.01322

* 20 cc aliquot of strip solution used.

Collaborators were requested to analyze both solutions by the colorimetric dithizone procedure, using either the photometer or Nessler tubes as preferred, and to check their results electrolytically either directly or after a dithizone extraction if manganese were found present. If the dithizone extraction was found necessary, collaborators were directed to use 15 cc. of 10 per cent potassium cyanide instead of the prescribed 5 cc. This was due to the presence of the large amount of zinc. Blanks of 0.0004 grain per pound on Solution A and 0.0017 grain per pound on Solution B were found by carrying 1400 grams of apples through the procedures without the addition of lead. Collaborators were requested to subtract these blanks from the indicated amount of lead. The procedures appear in the 1935 edition of *Methods of Analysis, A.O.A.C.*, under Chapter **29**, pars. **30**, **31**, **32**, and **33**. Results are given in Table I.

DISCUSSION OF RESULTS

Colorimetric Method.— With reference to Solution A, results range from 0.016 to 0.0186, with an average of 0.0179. On Solution B the results indi-

cated by an asterisk were obtained with a 20 cc. aliquot of strip solution, and all are low. The other results, with the exception of those of Collaborator 5, were obtained by the photometric method, which prescribes a 10 cc. aliquot. Collaborator 5 used a 10 cc. aliquot and the Nessler tube method, multiplying his results by two.

Two of the three collaborators using the 20 cc. aliquot complained of a heavy turbidity in the aqueous fraction of the color mixture. This precipitate is, of course, the source of the difficulty, as lead is bound up by occlusion and is not extracted by dithizone. The precipitate itself was identified as silicate. Additional portions of Solution B were sent to Collaborators 3, 7, and 12, and they were requested to repeat the colorimetric determination, using a 10 cc. aliquot. The results appear in Table 1 below those indicated with an asterisk, and are the ones included in the average. The use of a 10 cc. aliquot cut the amount of interference in half, and better results were obtained in spite of the fact that experimental error was doubled. All three collaborators report results somewhat high by this procedure, and their figures bring the general average to somewhat over the true lead content. It thus appears that the amount of silica which can be tolerated in the determination is about 50 mg. per 1400 grams of apples. In the presence of normal amounts of the alkaline earth metals, much more may be tolerated.

In this connection it may be well to recount some experiments undertaken by the Referee (June 1934) upon this same question of silicate interference. Increasing amounts of commercial sodium silicate (40° B) were added from a weight buret, along with an amount of lead equivalent to 0.019 grain per pound, to a succession of 500 cc. flasks containing the alkaline strip solution of 10 lead-free apples each. With the Nessler tube procedure and a 20 cc. aliquot, it was found that 1.0 gram of the commercial sodium silicate could be added before a precipitate appeared in the aqueous layer of the Nessler tubes, although 0.7 gram caused a haziness. Readings were not significantly low, however, until over 1.5 grams had been added. To further test the effect of silicate, two batches of commercial apples, silicate-washed but unrinsed, were obtained. These apples were covered with a chalky deposit and were in an entirely unmarketable condition; 1400 grams of one lot was stripped, hydrochloric acid being used for rinse and acidification, and analyzed with the following results: colorimetric dithizone with 20 cc. aliquot, 0.014+ (4 persons); electrolytic after a dithizone extraction, 0.0144. The result obtained by drying down and ashing a 100 cc. aliquot of the alkaline dip solution, extracting, and determining electrolytically, was 0.0135 grain per pound. With a nitric acid rinse and acidification, corresponding results upon another sample of the same lot were: colorimetric (4 persons), 0.013; electrolytic (direct), 0.0127; electrolytic after ashing and extracting, 0.0135 grain per pound.

With the other lot results were: colorimetric (10 cc. aliquot), 0.040;

electrolytic (direct), 0.0375; ashing, 0.0383. Other lots of commercial silicate-washed apples were analyzed in a similar way with closely agreeing results by the various methods, and in no case was interference noted. No precipitate appeared in the sample tubes in any case.

It thus appears that interference from silicates in the colorimetric dithizone method is extremely unlikely. In any event, the analyst is always warned by the appearance of a mineral precipitate in his tubes, and can so choose his aliquot as to limit this interference.

Electrolytic Method.—The direct electrolytic results on Solution A are, on the whole, satisfactory, ranging from 0.016 to 0.0186. On Solution B, where the dithizone extraction was necessary, results tend to be slightly low on the average due once more, undoubtedly, to the influence of silicate in the extraction. Here, more citric acid than that prescribed in the method might have helped.

During the past several years it has been the custom of commercial chemists in the Northwest to collaborate with the Seattle Station of the Food and Drug Administration and with one another in sending out "check" solutions, which are analyzed by each laboratory. The large amount of data compiled shows very good agreement among analysts using the colorimetric dithizone procedure. Some of these data are presented here. It is realized that good agreement among analysts with a given method is not an evidence of accuracy, but the fact that the electrolytic results* also recorded agree so closely with the general colorimetric average makes these figures very strong secondary evidence of the accuracy of both methods, especially when it is realized that they are entirely different in principle. These figures, in no way selected, are chosen as being typical. Many more could be presented from other sources showing as good agreement between the two methods.

Zinc does not seem to cause a serious interference in either method. It seems extremely unlikely that amounts of zinc larger than that added as an interference in this study will ever be encountered in actual practice. Cases where zinc has been reported to interfere are ascribed to the use of old potassium cyanide that has suffered deterioration.

Reports of interference due to the influence of sulfur sprays and sulfur-containing insecticides are more persistent. This interference is stated to occur when hydrochloric acid is used for acidification of the alkaline strip solution, as lead which may have been precipitated as sulfide is said to be only partially redissolved by this acid. This question is not answered by the collaborative results reported here, but it can be definitely stated that no interference occurs from sulfur when nitric acid is used. It may be said, however, that the Seattle Station of the Food and Drug Administration has investigated this question, working with lime-sulfur and other sulfur-bearing sprays, and has found no reason for recommending that

* By F. D. Clarke, Seattle Station, Food and Drug Administration.

TABLE 2.—*Comparison of colorimetric and electrolytic results (1936)**

SAMPLE NUMBER	COLORIMETRIC DITHIZONE			ELECTROLYTIC
	AVERAGE	MAXIMUM	MINIMUM	
		grain per pound		
1	0.0146	0.015	0.014	0.0139
2	0.0104	0.012	0.010	0.0104
3	0.0127	0.014	0.011	0.0124
4	0.0186	0.020	0.017	0.0190
5	0.0172	0.020	0.015	0.0175
6	0.0165	0.018	0.015	0.0180
7	0.0103	0.011	0.009	0.0094
8	0.0202	0.022	0.020	0.0209
9	0.0078	0.009	0.007	0.0081
10	0.0143	0.016	0.013	0.0143
11	0.0200	0.021	0.019	0.0202
12	0.0162	0.017	0.016	0.0163
13	0.0140	0.016	0.013	0.0144

* With the exception of Samples No. 1 and 2, in which 5 laboratories collaborated, 26 laboratories participated in the collaborative work.

nitric acid, exclusively, should be used for acidification. More time will be given to this question, but at present there seems to be no reason for abandoning the use of hydrochloric acid if analysts desire to use it.

The mush-dithizone extraction and electrolytic procedure, par. **29**, has been used extensively by the Bureau of Entomology and Plant Quarantine in its investigations, and exhaustive recovery experiments have proved its reliability. However, very few collaborative data are available. It is hoped that some can be offered next year.

GENERAL

The method of dithizone isolation of lead, preparatory to an electrolytic determination, par. **16(a)**, calls for an evaporation of the dithizone extracts, digestion of the residue with concentrated nitric acid, dilution, and adjustment of the acidity to 1 per cent by neutralization with ammonium hydroxide and the addition of the proper amount of nitric acid. The same purpose can be attained by "stripping" the lead from the dithizone extracts with 1 per cent nitric acid and directly electrolyzing an aliquot. This would undoubtedly save much time and manipulation, and this shorter procedure has been proposed by several analysts during the past three years. While this "short cut" method is attractive, the Associate Referee has avoided it because of the possibility of chloride development under the conditions of electrolysis, and experiments conducted under date of October, 1934, showed slightly low recoveries by this technic. However, more recent data have shown that the lead loss, if any, is very small; this being the case, no objection can be made to its use, at least for routine work.

More disquieting are reports of lead losses in the ashing at the prescribed 500° of high carbohydrate foods, notably maple sugar. These reports are undoubtedly well founded, and this question must be investigated. The same difficulty has arisen with the lead determination in baking powders and will be dealt with in more detail in that report.

The removal of interferences as outlined in the present tentative method is admittedly laborious and time-consuming. For this reason a suggestion from Grigsby and Berman* that cupferron be used was very welcome. Some work was done in which the iron (which oxidizes dithizone in the alkaline extraction of lead) and tin (which is likely to precipitate out and occlude lead under these conditions, or to contaminate the electrode deposit in the subsequent electrolysis) were precipitated out with a cupferron solution and removed by an ether extraction. Large amounts of both metals can be eliminated in this way. The precipitation is made directly upon the acid solution of the ash. Bismuth is also partially removed (80 per cent), and lead is not lost under these conditions. In preliminary work excellent recoveries of small amounts of lead in the presence of as much as 100 mg. each of iron and tin were obtained. This procedure will be collaboratively studied during the coming year.

The use of hydroxylamine hydrochloride to prevent oxidation of dithizone, as proposed by Winkler, *This Journal,* **19**, 233 (1936), and others, aids alkaline dithizone extraction of lead, and the use of 2–5 cc. of a 1 per cent solution of the salt is recommended whenever oxidation of dithizone, due either to iron or organic oxidants, occurs.

Other work done by the Associate Referee during the past year involved a study of the permanency of dithizone solutions. Stable solutions will increase the accuracy of the present dithizone methods, especially for small amounts of lead, and may make possible the detection and perhaps even the co-determination of the interferences of bismuth, tin, and thallium along with lead. Results obtained are as yet incomplete, but it may be said that the predominant cause of deterioration with both chloroform and carbon tetrachloride solutions of dithizone is heat. Deterioration occurs much more rapidly during the summer months, and it was found that dithizone solutions in chloroform will keep almost indefinitely if stored in an ice box. No known method of preserving chloroform solutions involving the use of sulfite, hydroxylamine, etc., has proved of any value, but it has been found that sulfurous acid *does* aid in the preservation of carbon tetrachloride solutions of dithizone. It is the Referee's thought, as borne out by experiment, that carbon tetrachloride may be substituted for chloroform, in the "mixed-color" dithizone method for lead, *provided* photometric methods are used. The reason is that carbon tetrachloride is unadaptable to visual mixed-color methods because excess dithizone in the mixed color goes almost entirely into the aqueous phase,

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* Food and Drug Administration, Philadelphia, Pa.

and the color series appears to be a succession of deeper and deeper pink colors. Thus the value of the "mixed-color" procedure, viz., the unique transition in hue from green to red, is largely lost to visual methods of comparison, but with photometric methods the "spread," and hence the sensitivity, in the green region of the spectrum, is enhanced. Work is in active progress and it is hoped that valuable suggestions may be made next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That the rapid method for the determination of lead, restricted to apples and pears, with either colorimetric or electrolytic estimation of lead, be made official.

(2) That work be continued on interference removal and permanency of dithizone solutions.

NOTE ON THE DETERMINATION OF LEAD IN SPRAY RESIDUE

By P. A. Clifford

Since the report of the Associate Referee on Lead was presented at the 1937 meeting of the Association, developments have apparently made desirable a modification of certain details of the colorimetric dithizone method. Pending formal action next year, opportunity is being taken at this time to suggest these changes.

As written in the last (1935) edition of *Methods of Analysis*, p. 391, par. **30**, either hydrochloric or nitric acid may be used for rinsing the apples and for acidification of the alkaline strip solution. In most instances hydrochloric has been used for this purpose, the advantage being that a Gutzeit arsenic determination may be run directly on an aliquot of the filtrate used for the colorimetric lead determination. In the Associate Referee's report it was noted that the use of lime-sulfur sprays causes some concern as being a possible source of low lead results when hydrochloric acid is used for acidification. This effect has been checked repeatedly with apples known to have been sprayed with lime-sulfur and no interference was noted, but in December, 1937, it was found that in one or two instances lime-sulfur in considerable quantities was being added directly to the washing tanks, and that if analyses were made within a period of two or three weeks by the colorimetric dithizone method, and hydrochloric acid used for acidification, low lead results were obtained. Serious repression of lead results was not noted after a longer period, presumably due to oxidation of the lime-sulfur complex. This would account for the fact that interference was not noted when the lime-sulfur was used as a spray earlier in the season.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 71 (1938).

Recent experiments, as well as those reported here, show that no interference occurs when nitric acid is used for acidification. It is unnecessary to heat the filtrate. For this reason, it is suggested, and it will be formally proposed next year, that nitric acid exclusively be used for acidification of the apple samples. It appears that hydrochloric acid may be retained in the rinse and that a rapid approximate Gutzeit arsenic determination may still be run on a further aliquot of the strip solution after acidification with hydrochloric acid, as the repression of arsenic does not appear to be as serious as that of lead. The hydrogen sulfide interference may be serious in the arsenic determination in only a few instances, and these should be detected by an odor of hydrogen sulfide in the filtrate or an undue blackening of the scrubber of the Gutzeit generator. So far as lead is concerned, there is no objection to the slight mixing of hydrochloric and nitric acids resulting from the use of a hydrochloric acid rinse and nitric acid acidification. The standard tubes may be made up with nitric acid alone or, if the analyst wishes, he may use 90 cc. of nitric acid and 15 cc. of hydrochloric acid per liter in the preparation of the "standard" and "blank" solutions, taking care to mix the acids only when the solution is cold and dilute. The elimination of lime-sulfur as an interference would leave no other substance likely to interfere in the colorimetric dithizone method for lead on apples.

It has been recently brought to the Referee's attention that low results may sometimes be obtained by the *direct* electrolytic method on aliquots of the acidified and filtered strip solution in those cases where excessive amounts of organic material have been incorporated in the dipping procedure. This situation may arise in the dipping of soft and badly bruised apples, or when too much sugar is included in the dipping solution by cutting out the stem and calyx ends of the apples so widely as to expose a considerable amount of the apple flesh. The directions call for the exposing of no more flesh than is necessary, and if care is taken in the trimming of stem and calyx ends, no trouble should be experienced. However, it is conceivable that such trouble may sometimes arise, either as the result of careless trimming, or in the analysis of "mushy" apples. Consequently (and in the face of the excellent results given in the regular report by direct electrolysis on Solution A, Table 1), the Referee favors the abandonment of the electrolytic procedure as applied directly to the apple filtrate. Instead, the electrolysis should be made only after dithizone extraction. This would render unnecessary the testing of the apple filtrate for the presence of manganese, as prescribed in the published method. This entire paragraph consequently will need revision, but this must be postponed until next year. The direct electrolytic method has formerly been recommended as a check on the colorimetric dithizone determination but the increased confidence that analysts have shown in the latter has necessitated little use of the electrolytic procedure. In the rare instances

where electrolytic check is indicated, little time is lost on account of the preliminary dithizone isolation of the lead, and the analyst is assured of obtaining the full amount of lead actually present in the solution.

REPORT ON MERCURY

By W. O. Winkler (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In the report of the Associate Referee on Mercury for 1935 (no report was made in 1936) it was stated that reagents often contain mercury, but methods of purification were not given. In addition, it was concluded that mercury could not be extracted with dithizone from dilute acid solutions containing organic matter. There was also expressed a desire to avoid the necessity of oxidizing all the organic matter in the sample.

The possibility of applying a photometric method was suggested by the wide difference in the light transmission curves of dithizone and the mercury-dithizone complex at a wave length of 610 mu.

Therefore, the work this year comprised the following problems:

I. Reagents and their purification.
II. Sample preparation and concentration of mercury.
III. Isolation.
IV. Determination.

REAGENTS

It is obvious that reagents of a high degree of purity are essential in determining micro quantities of an element. Although formerly it was thought that nitric acid contained no mercury, some supplies have been found to contain small quantities. The acid can be purified by distillation over concentrated sulfuric acid, since mercuric sulfate is not volatile at the temperature of the distillation.

Sulfuric acid can be freed from mercury, if necessary, by dilution with water (1:10) and extraction with diphenylthiocarbazone (dithizone) Reducing agents and neutral salt solutions can also be purified in this way. Potassium permanganate has been found to contain small quantities of mercury, but there appears to be no satisfactory method of purifying this reagent.

SAMPLE PREPARATION AND CONCENTRATION OF MERCURY

The former finding that organic matter inhibits the extraction of mercury from dilute acid solution with dithizone was confirmed. Oxidation is the most time-consuming part of the determination, and it was thought that this procedure could be shortened if the mercury was concentrated first with a minimum of organic matter, which could later be readily oxi-

dized. To accomplish the concentration, three precipitation methods were tried.

The first of these methods was the precipitation of mercury as metal or oxide from weakly acid, neutral, or alkaline solutions. Formaldehyde was used as the reducing agent, and the reaction was carried out on the steam bath. Barium sulfate was used first as the gathering agent, and later alumina cream was tried. The gathering agent was separated by centrifuging. The results have not been satisfactory. The maximum recovery was 50 per cent, obtained from an alkaline solution containing 100 gamma (0.1 mg.) of mercury. Next year the Associate Referee intends to try precipitated ferric hydroxide as the mercury adsorbent with an additional reducing agent.

Precipitation of the mercury as the sulfide was the second method of concentration tried. A few drops of 5 per cent stannous chloride were added to aid in gathering the precipitate. Hydrogen sulfide was passed through the solution for 10–15 minutes in a centrifuge bottle, and the precipitated sulfides were separated by centrifuging. Alumina cream was added in some cases to aid in collecting the mercuric sulfide precipitate from the solution. A maximum of 60 per cent was recovered by this procedure. It appears that a longer time is necessary to effect complete precipitation or separation of the mercuric sulfide from solution when only small amounts are present.

Precipitation by the addition of powdered metal was the third method of concentration tried. Iron, aluminum, and zinc were used for this purpose. Zinc proved to be much superior to the other two metals. A coating of oxide or adsorbed air appeared to prevent intimate contact of the aluminum with the solution, and very little mercury was precipitated by this metal. Iron required a much larger quantity of acid and was difficult to dissolve completely. The zinc presented no such problems although it was found to contain a small amount of mercury.

To establish the optimum conditions for precipitation, five samples of mercury in nitric acid solution, each containing 0.1 mg. (100 gamma) of mercury and 10 cc. of nitric acid, were subjected to the following treatments: To No. 1 was added 10 cc. of concentrated ammonium hydroxide to partially neutralize the nitric acid; to No. 2 was added 12 cc. of concentrated ammonium hydroxide, sufficient to make the solution slightly alkaline; to No. 3 was added 12 cc. of concentrated ammonium hydroxide and then sufficient hydrochloric acid (1+1) to provide an excess of 2.5 cc.; No. 4 was made alkaline with ammonium hydroxide and then just acid with hydrochloric acid; and No. 5 was made alkaline with concentrated ammonium hydroxide and then slightly acid with nitric acid.

All samples were diluted to a volume of 150 cc. and shaken with 1.5 grams of powdered zinc, and the metal was separated by centrifuging. After the supernatant liquid had been decanted, the residue was dissolved

in hot nitric acid (1+1) and diluted; nitrites were removed with hydroxylamine-hydrochloride, and the mercury was determined by titration. Results are given in Table 1.

The results indicate that the optimum conditions for precipitation with zinc are found in a dilute hydrochloric acid solution, probably be-

TABLE 1.—*Recovery of mercury by precipitation with powdered zinc at different acidities*

SAMPLE NO.	TREATMENT	TITRATION	RECOVERY
			per cent
1	3.5 cc. excess HNO_3	Too acid, no value obtained	
2	Slightly ammoniacal	5.6	74.8
3	2.5 cc. HCl (1+1) in excess	7.21	101.6
4	Slightly acid HCl	6.81	95.9
5	Slightly acid HNO_3	6.57	92.5

tween pH 1 and 2. The zinc reacts partially with the acid under these conditions, forming what appears to be an oxychloride, which assists in collecting the precipitated mercury. The value of 101.6 per cent obtained was not corrected for reagent blank and would probably be slightly below 100 per cent after this correction.

The method was then applied to nitric acid extracts of leafy vegetables containing added mercury and also to larger quantities of mercury

TABLE 2.—*Recovery of mercury by precipitation with zinc from nitric acid extracts of lettuce in presence of HCl and H_2SO_4*

SAMPLE	ACIDITY	MERCURY ADDED	MERCURY FOUND	REAGENT AND LETTUCE BLANK	RECOVERY
		gamma	*gamma*	*gamma*	*per cent*
HNO_3 extract of lettuce	2 cc. excess HCl (1+1) in 160 cc.	50	63.7	22.2	88*
HNO_3 extract of lettuce	Slightly acid to litmus H_2SO_4	50	51.4	13.7	80.5*
HNO_3 extract of lettuce	1 cc. excess of H_2SO_4 (1+4)	50	60.1	13.7	94
HNO_3 extract of lettuce	2 cc. HCl (1+1) in 160 cc.	50	59.6	9.3	100.4
Acid salt solution	2 cc. HCl (1+1) in 160 cc.	1000	1012.	8.	100.5

* Zinc agglomerated or stuck to bottom.

(1 mg.) than had previously been tried. As a comparison, precipitations were also made from dilute sulfuric acid solution.

Results of these experiments are given in Table 2.

An examination of Table 2 shows that 100 per cent recovery of mercury can be obtained. The high blanks on some of the samples apparently were

due to the lettuce, as the zinc gave a mercury content of only 6 or 7 gamma on a 1.5 gram sample, which was the quantity used for precipitation. The low result of 88 per cent on the first sample, when hydrochloric acid was used, was due in all probability to the zinc sticking to the bottom of the bottle and agglomerating to some extent, thereby preventing intimate contact with the solution. This also occurred in some other experiments, especially if the bottles stood before they were shaken after addition of the zinc. It is very necessary, therefore, to keep the zinc in a fine state and to prevent it from adhering to the bottle. To accomplish this the bottom of the bottle was first covered with a layer of ordinary sea sand. Shaking was begun immediately upon the addition of the zinc and continued for 7 or 8 minutes. If intimate contact is obtained, there is no apparent reason why complete precipitation should not follow. A small amount of organic material adheres to the zinc precipitate, and it is necessary for best results to oxidize it with potassium permanganate after dissolving the zinc in nitric acid (1+1).

The procedure for zinc precipitation, which may be modified later, is as follows:

Neutralize the HNO_3 extract of the lettuce (obtained as directed in the reports for former years) with concentrated NH_4OH and place in centrifuge bottles. Run a quantity convenient for shaking (about 160 cc.) in each 250 cc. bottle and make the solution just acid with HCl (1+1). Add 2 cc. in excess and sufficient sand to cover the bottom about ¼" deep (9 or 10 grams), and then add 1.25 grams of powdered zinc. Stopper the bottle and immediately shake for 7–8 minutes. Separate the precipitate from the liquid by centrifuging 8–10 minutes at 1800 r.p.m. Decant and discard the supernatant liquid. Add to the precipitate 12 cc. of HNO_3 (1+1), and when the reaction has subsided place the covered sample on the steam bath for 5–10 minutes. Transfer the solution to the digestion flask and after dilution to about 100 cc. add 3 grams of permanganate; mix, and heat to gentle boiling for a few minutes. The purple color of the permanganate should remain. If there is no purple color left, add more $KMnO_4$. Reduce the hot $KMnO_4$ solution (above 70° C. but not boiling) by adding slowly, while shaking, a saturated solution of $FeSO_4 \cdot 7H_2O$ in 1% H_2SO_4.

ISOLATION OF MERCURY

A more rapid and satisfactory method of isolating the mercury than that previously used was found. After the oxidation of the organic matter as described previously, the procedure is as follows:

Extract the mercury with an excess of the dithizone extraction solution. Repeat with one extra portion of the extractant. Shake the combined extracts with a solution containing 75 cc. of water, 2 cc. of H_2SO_4 (1+1), and 4 cc. of 1.5% $Na_2S_2O_3$ solution. Withdraw the lower $CHCl_3$ layer and wash the acid aqueous layer with about 10 cc. of $CHCl_3$. Discard the $CHCl_3$ extracts. (The mercury is transferred to the aqueous layer by the stronger attraction of $Na_2S_2O_3$.) Place the aqueous solution in a 250 cc. beaker and add 7 or 8 cc. of saturated $KMnO_4$ solution. Mix, and allow the solution to stand 8–10 minutes at room temperature. Clear the solution by adding dropwise, with stirring, a 5% solution of $NH_2OH \cdot HCl$, and add 0.7 cc. in

excess. Warm the solution to 53°–56° C., cool, and determine Hg by one of the three methods given under "Determination of Mercury."

The above procedure serves not only to remove other metals but it also produces a solution that maintains the dithizone in stable condition during the determination.

DETERMINATION OF MERCURY

(A) *Factors affecting the determination.*—Before considering the actual determination it is necessary to present some factors that affect the mercury-dithizone reaction.

The quantitative relation of mercury to dithizone in the mercury-dithizone complex has been found to change with certain conditions and constituents of the solution from which the mercury is extracted.

Helmut Fischer[1] has advanced the theory that there are two forms of the mercury dithizone complex, namely, the enol and keto. According to his theory the enol form, produced in alkaline solution, is composed of one molecule of dithizone and one atom of mercury. The keto form, he holds, is present in acid solution and is composed of two molecules of dithizone and one atom of mercury. Mixtures of the two forms may exist under varying conditions.

The theory that two forms exist is undoubtedly correct. It shows the necessity of making the determination under standard conditions. The Associate Referee has been unable to confirm the theory that acidity or alkalinity is the controlling factor in the formation of the two complexes, especially the postulate that the enol form is produced in alkaline solution. With equal quantities of mercury, larger titrations have been obtained with ammoniacal solutions than with sulfuric acid of 0.75 *N* strength. A lower titration should have been obtained in case of enol formation. Moreover, substantially the same titration was obtained with three samples containing equal quantities of mercury, one acid with sulfuric acid, the second neutral to litmus, and the third alkaline with sodium hydroxide. This would tend to show that *p*H is not the potent factor in the production of the two forms.

Certain complex formers that have been found to exert a decided influence on the reaction are NH_4OH, $NH_2OH \cdot HCl$, KI, KBr, Na_2SO_3, and $Na_2S_2O_3$. The $Na_2S_2O_3$ and KI prevent the dithizone reaction in acid titration by forming stronger complexes. Hydroxylamine has been found to favor a higher ratio of dithizone to mercury. Some of these results are given in Table 3.

The results indicate that the *p*H of the solution is not the only factor affecting the dithizone reaction, and they also show the necessity of making the determination under definite conditions.

(B) *Methods.*—Work was done this year on two methods of determina-

[1] *Z. anal. Chem.*, 103, 241 (1935).

TABLE 3.—*Titrations of mercury by extraction with dithizone from solutions of different composition*

SAMPLE NO.	MATERIALS PRESENT IN SOLUTION	WATER	MERCURY ADDED	TITRATION
		cc.	*gamma*	
1	2 cc. H_2SO_4 (1+1)	50	100	14.6
2	1 cc. NH_4OH+0.7 gram NH_4 citrate	50	100	19.9
3	2 cc. H_2SO_4 (1+1)+4 cc. 15% $NH_2OH \cdot HCl$	50	100	16.4
4	2 cc. H_2SO_4 (1+1) 2 cc. 10% $NaNO_2$ +2.5 cc. 30% H_2O_2 heated	50	100	14.7
5	$(NH_4)_2SO_4$ neutral to litmus	50	100	14.6

tion besides the titrametric method. They were the comparative method used by Fischer (*loc cit.*) and a photometric method similar to or adapted from that used by Clifford and Wichmann for lead, *This Journal*, **19**, 130 (1936).

The procedure for the determination by duplication or comparison, which is a modification of Fischer's method, is as follows:

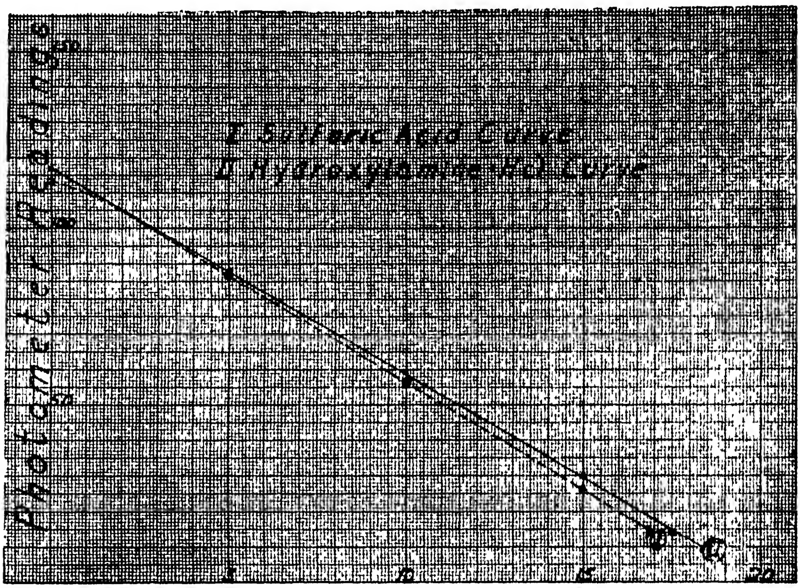
To the separatory funnel containing the mercury solution obtained in the isolation procedure, add sufficient dithizone solution (12.5 mg./l.) accurately measured to provide an excess. (The addition may be made in several portions until the excess is reached, all the solution being left in the funnel.) Shake vigorously for about 30 seconds, allow the liquids to separate, and withdraw the dithizone layer with some of the acid layer to a Nessler tube. To a second glass-stoppered Nessler tube, which should match the first one, add a little dilute H_2SO_4 solution, 0.7 cc. of 5% $NH_2OH \cdot HCl$ solution, and a volume of dithizone equal to that used in the sample. Add to this Nessler tube from a 10 cc. buret a standard solution of Hg (10 gamma per cc.), prepared as directed in *This Journal*, **18**, 64 (1935), shaking frequently, until the colors of the dithizone layers match. The Hg added is equal to that in the sample. The strength of the dithizone solution may be varied according to the quantity of Hg it is desired to determine.

An accuracy approaching 1 gamma was obtained by use of this method, which agrees with the findings of Fischer and is of the same order as the results obtained by the titrametric method proposed by the Associate Referee. Some results are given in Table 4.

TABLE 4.—*Determination of mercury by duplication*

MERCURY ADDED	MERCURY FOUND	ERROR
gamma	*gamma*	*gamma*
1.1	1.0	−0.1
18.2	17.0	−1 2
0	0	0
80.6	83	+2.4
46.7	46.0	−0.7
100	101	+1.0
16.9	16.5	−0.4

The work on a photometric method has progressed very satisfactorily although considerable difficulty was met at first. The photometer used was the same as that used by Clifford and Wichmann (*loc. cit.*) for the photometric determination of lead. A photograph and a description of the instrument will be found in their publication. For the work this year the Associate Referee used a light filter centered at about 610 mu. This wave length was chosen because it gives the greatest spread between the light transmissions of the free dithizone and the mercury-dithizone com-



GAMMA OF MERCURY

FIG. 1.

plex. As this wave length is in the yellow or orange portion of the spectrum, the light is transmitted by the mercury complex and absorbed by the green dithizone. The process, therefore, becomes one of measuring the absorption of the uncombined dithizone. Obviously, the solution from which the mercury is extracted with dithizone must be such as to maintain the dithizone in as stable a condition as possible, because any oxidation product gives a lower absorption in the photometer and therefore gives results which are too high. Sulfuric acid solutions are better than nitric acid for this purpose. If the extractions are made from dilute sulfuric acid (about 1%) a linear curve is obtained when photometer readings as ordinates are plotted against gamma of mercury as abscissas, except for a small drop at the top.

It is not possible in the actual treatment of a sample to arrive at the point of determination, with the mercury in a plain sulfuric acid solution. After considerable experimentation the procedure given under "Isolation

of Mercury" was selected as approaching the nearest to this ideal. The determination was made by extracting the mercury with 5 cc. of a chloroform solution containing 10 or 12.5 mg. of dithizone per liter (prepared by diluting a stronger stock solution). Readings were made in the photometer by means of a half-inch cell. (Solutions stronger than 12.5 mg. per liter are difficult to read.) By comparing the reading with a standard curve prepared with known quantities of mercury on the same dithizone solution, the amount of mercury may be read directly. Amounts from 0 to 18 gamma may be determined by using 5 cc. of dithizone solution containing 10 mg. per liter. For larger quantities a larger volume must be used, but there is a corresponding drop in sensitivity. Some results by the photometric method on samples of which the analyst did not know the mercury content when making the determination are given in Table 5. Formic acid and hydroquinone were used as the reducing agents in these determinations.

These reducing agents and a number of others were tried out before hydroxylamine hydrochloride was adopted. Time has not permitted experiments on recovery since starting the use of hydroxylamine but the results should be more accurate.

TABLE 5.—*Results of mercury determinations by the photometric method with formic acid and hydroquinone as the reducing agents*

MERCURY PRESENT	MERCURY FOUND	ERROR
gamma	*gamma*	*gamma*
0.8	2	+1 2
4.8	5.5	+0 7
29.7	30 0	+0 3
16 7	16.5	−0.2
12.3	12 0	−0.3
8.8	9.0	+0.2

Examination of the table shows recoveries which were too high in the range from 0 to 5 gamma. This would tend to show that a slight oxidation occurred. Results on other portions of the curve give some indication of the possibilities of the method.

Curves obtained by plotting photometer readings against gamma of mercury, when the extractions were made from dilute sulfuric acid, and from solutions reduced with hydroxylamine hydrochloride (as given under isolation of mercury), are given in Fig. 1. The hydroxylamine curve is superior to the plain acid curve in the portion below 5 gamma, and by its use the difficulty above should be largely overcome.

RECOMMENDATIONS[1]

It is recommended—

1. That work be continued on the following subjects: zinc precipitation

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 21, 70 (1938).

method of isolation, the photometric method of determination, and the formaldehyde method of isolation.

(2) That collaborative work be done on the titrametric and colorimetric methods for mercury determinations described in this report.

REPORT ON SELENIUM

By A. L. CURL and R. A. OSBORN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year the selenium report, *This Journal*, **20**, 194 (1937), suggested further study of methods of sample preparation and determination. Such studies were made, and it is believed that the procedure has been simplified without sacrifice to accuracy. The method of isolating selenium is so well established that no study of this step was made. The volumetric measurement of selenium was further studied and the reproducibility of the titration with starch as indicator was compared with results obtained by using the electrometric method, also published in last year's report. These studies indicate that the use of starch simplifies the method. The results are of about the same degree of accuracy.

STUDIES WITH THE PUBLISHED PROCEDURE

Variations in weight of sample for analysis, and effect of a mercury catalyst.—The published procedure was used for the analysis of a standard sample of mixed seleniferous vegetation composed of ground sunflower,

TABLE 1.—*Summary of results on effect of variation in weight of sample*

SAMPLE	Se FOUND	
grams	*p.p.m.*	*av. p.p.m.*
10	167	
10	166	166
5	164	
5	164	164
2	167	167
1	164	
1	156	160
5 No mercury catalyst	9	
5 No mercury catalyst	22	16

wheat, barley, and oats, having a selenium content of approximately 165 p.p.m. Duplicate 10, 5, 2, and 1 gram samples were analyzed. Table 1 summarizes the results obtained and also gives results of analysis of duplicate 5 gram portions of the sample digested without the use of the mercury catalyst. The titrations were made with 0.01 *N* standard thiosulfate and iodine solutions.

From these data (Table 1) it appears that the weight of sample taken had no significant effect on the values obtained. It seems logical to assume that significant losses of selenium did not occur with this procedure and this sample. If losses occurred, it would be necessary to assume that they were directly proportional to the weight of sample taken.

The necessity of using the mercury catalyst during the digestion is strikingly demonstrated, for without it the selenium loss is approximately 90 per cent.

TABLE 2.—*Results of a study of wet digestion procedures of sample preparation (Duplicate, 5 gram amounts of a standard sample)*

	Procedure employed during digestion	Se obtained *p.p.m.*
1.	Mathews, Curl, and Osborn, *This Journal*, **20**, 201 (1937)	164, 164
2.	Same as 1, except no mercury catalyst added	9, 22
3.	Williams and Lakin, $HNO_3+H_2SO_4$, no catalyst and temperature not above 120° C. *Ind. Eng. Chem. Anal. Ed.*, **7**, 409 (1935)	163, 165
4.	Same as 3, except digestion on steam bath (less than 100° C.)	163, 163
5.	Same as 3, except digestion prolonged to brown color and 100cc. HNO_3 used	138, 149
6.	Same as 3, except digestion prolonged to brown color and 50 cc. HNO_3 used	158,—
7.	Simplified procedure in 600 cc. beaker, see p. 234	164, 164
8.	Simplified procedure in Kjeldahl flask, see p. 234	168, 164
9.	Same as 7, except complete digestion in 600 cc. beaker	161, 164
10.	Same as 8, except complete digestion in Kjeldahl flask	164, 164
11.	50 cc. H_2SO_4, 0.5 g. HgO (no HNO_3), complete digestion in Kjeldahl flask	149, 151
12.	50 cc. H_2SO_4, 1.0 g. $CuSO_4$ (no HNO_3), complete digestion in Kjeldahl flask	123, 130
13.	100 cc. conc. HBr, 15 cc. Br_2 under reflux 1½ hr.	130, 132

Effect of variations in wet digestion procedure.—A number of wet digestion procedures of sample preparation were studied. Duplicate 5 gram amounts of the standard selenium vegetation sample referred to in Table 1 were taken and digested as described in Table 2. After digestion the samples were subjected to the published procedure of isolation and electrometric titration.

It will be observed (Table 2) that at least seven of the procedures of digestion give selenium values that are in good agreement, namely, Nos. 1, 3, 4, 7, 8, 9, and 10. Procedure 4 requires about 3.5 hours for digestion. The distillate contains waxy material and the residual nitrogen compounds may liberate considerable free bromine during distillation. Procedure 3 requires about two hours for completion of digestion, and care is necessary in regard to temperature control. This procedure, likewise, gives rise to waxy material and an excess of free bromine, but to a lesser extent. Procedures 7 and 8 were preferred by the writers because less time

is required for digestion than by Procedures 3 or 4. No material amount of bromine is liberated during the distillation and smaller amounts of waxy material are obtained. Procedures 9 and 10 require several hours for completion of digestion. There is a minimum of waxy material on distillation.

In carrying out any of these procedures it is necessary to avoid having the digestion proceed too rapidly at the start, since too violent a reaction may lead to some loss of selenium. The digestion procedures believed to be preferable (7, 8, 9, 10) differ from an ordinary wet digestion with nitric and sulfuric acid in one important respect, the presence of the added mercury. This difference is essential if the analyst wishes to digest samples quickly and without the necessity of a careful control of the temperature.

TABLE 3.—*Analysis of selenium vegetation samples*

PROCEDURES AS DESCRIBED IN TABLE 2	SELENIUM (p.p.m.)			
	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
1	164, 164	68, 70	40, 40	39, —
3	163, 165	72, 73	44, 45	44, 45
4	163, 163	72, 74	41, 44	44, 45
7	164, 164	72, 73	43, 43	44, 45
10	164, 164	71, 73	42, 43	42, 42

Both mercury and selenium are better catalysts for the oxidation of organic material than is copper. Mercury reduces the volatility of selenium, but copper does not appear to exhibit this effect. This behavior is in accord with observations by Osborn and Krasnitz in their studies of the Kjeldahl method, *This Journal*, **17**, 339 (1934). The necessity of using the mercury during a complete digestion is also shown in Table 1. When a sample of selenium vegetation (No. 2) was analyzed by Procedure 7 (partial digestion with mercury), the selenium content was found to be 72 and 73 p.p.m. for 5 gram duplicate quantities. When the analysis was carried out in exactly the same manner but with omission of the mercury, values of 16 and 17 p.p.m. of selenium were obtained. This represents a loss of approximately 80 per cent.

Five of the procedures described in Table 2 (1, 3, 4, 7, 10) were used in the analysis of three additional standard samples of selenium vegetation. Duplicate 5 gram quantities of the samples were taken. After digestion the selenium was distilled and titrated electrometrically. Table 3 gives the results obtained. They are in good agreement. From the standpoint of the analytical values obtained, there does not appear to be a marked superiority in any one of the procedures. The values obtained by the complete digestion procedures (1, 10) are slightly lower than those obtained by the three incomplete digestion procedures.

In the belief that the lower results obtained by Procedure 1 might be due to too rapid digestion, these determinations were repeated at a later date, and digestion was carried on so slowly that it was completed before SO_3 fumes appeared. Selenium was determined volumetrically with starch as indicator. The results, expressed as p.p.m., indicate that a slower digestion gives somewhat higher figures. The results follow: Sample II, 76, 77; Sample III, 46, 47; Sample IV, 46, 46. Study of this matter will be continued.

Comparative study of analytical procedures.—Table 4 gives comparative results obtained by analyzing eight samples of selenium-containing vegetation by the Williams and Lakin procedure, and by digestion procedure 8 and the writers' published distillation and electrometric titration procedures.

TABLE 4.—*Comparative results*

SAMPLE	SELENIUM FOUND (p.p.m.)			
	WILLIAMS-LAKIN PROCEDURE*		WRITERS' PROCEDURE†	
A	1	Turbidimetric	1.12	1.08
B	70 ± 10	Gravimetric	64.0	65.2
C	150 ± 10	Gravimetric	162	163
D	70 ± 10	Gravimetric	67	64
E	230 ± 30	Gravimetric	215	213
F	270 ± 10	Gravimetric	264	262
G	170 ± 10	Gravimetric	169	166
H	180 ± 10	Gravimetric	173	178

* K. T. Williams, Analyst.
† A. L. Curl, Analyst.

It will be observed (Table 4) that the values obtained by the two procedures are in good agreement. The data are insufficient for comparison of the turbidimetric results with the volumetric. Last year's report however clearly indicates the general superiority of the volumetric over the turbidimetric procedure.

VOLUMETRIC MEASUREMENT OF SELENIUM—USING STARCH INDICATOR

Realizing that an electrometric titration apparatus may not be readily available to all chemists interested in the determination of small quantities of selenium, the writers considered it to be desirable to study the limitation of the titration with starch as indicator. It was found convenient to use the electric stirring device, the dipping buret, and 30 or 50 cc. beakers for the titration. The end point could be conveniently approached from either side when 0.01 N iodine and thiosulfate solutions were used. It was considered preferable, however, to add an excess of standard thiosulfate to reduce the selenium, then to add a slight excess of standard iodine, and

TABLE 5.—*Volumetric determination of selenium (gamma) with starch indicator*

Se IN UNKNOWNS	FOUND	DEVIATION	Se IN UNKNOWNS	FOUND	DEVIATION
0.01 N thiosulfate and iodine—10 ml. burets					
0	2	+2	110	110	0
0	−2	−2	145	144	−1
5	8	+3	190	194	+4
7	10	+3	260	260	0
10	8	−2	290	288	−2
15	16	+1	395	397	+2
19	14	−5	505	499	−6
26	18	−8	585	585	0
30	30	0	706	703	−3
37	38	+1	815	817	+2
39	44	+5	896	899	+3
46	44	−2	983	981	−2
49	48	−1	1175	1170	−5
55	55	0	1350	1358	+8
63	61	−2	1591	1590	−1
71	69	−2	1832	1837	+5
78	71	−7			
85	84	−1	Average Deviation		2.6
86	87	+1	Median Deviation		2
98	101	+3	Maximum Deviation		8
0.001 N thiosulfate and iodine—10 ml. burets					
0.0	+0.8	+0.8	19.8	19.3	−0.5
0.0	−0.6	−0.6	23.0	22.4	−0.6
0.3	−0.2	−0.5	31.0	31.2	+0.2
0.7	0.6	−0.1	40.0	39.2	−0.8
1.0	1.2	+0.2	48.0	48.8	+0.8
1.5	2.2	+0.7	59	59	0.0
1.9	2.0	+0.1	69	68.5	−0.5
3.0	2.4	−0.6	78	77.5	−0.5
4.0	3.7	−0.3	89	88.9	−0.1
5.7	5.7	0.0	100	101.6	+1.6
7.0	6.5	−0.5	121	121.0	0.0
9.1	9.1	0.0	139	139.1	+0.1
10.4	9.5	−0.9	158	157.7	−0.3
11.0	10.2	−0.8	177	175.8	−1.2
14.0	13.6	−0.4	191	191.4	+0.4
14.3	14.0	−0.3			
15.0	16.1	+1.1	Average Deviation		0.5
15.7	15.0	−0.7	Median Deviation		0.5
18.0	16.8	−1.2	Maximum Deviation		1.6
19.0	19.2	+0.2			

TABLE 5.—(*Continued*)

Se IN UNKNOWNS	FOUND	DEVIATION	Se IN UNKNOWNS	FOUND	DEVIATION
0.0005 N thiosulfate and iodine—10 ml. burets					
0.0	0.2	+0.2			
0.3	0.1	−0.2			
0.7	0.8	+0.1			
1.0	1.0	0.0			
1.6	1.3	−0.3			
2.0	2.1	+0.1			
2.9	2.7	−0.2			
4.0	4.0	0.0			
4.8	4.6	−0.2			
6.1	6.0	−0.1	Average Deviation		0.2
7.9	7.3	−0 6	Median Deviation		0.2
9.9	9.7	−0 2	Maximum Deviation		0 6

finally titrate back with standard thiosulfate to the point of the disappearance of the blue color. This method of approaching the end point was found to be more suitable for quantities of selenium titrated with 0.001 or 0.0005 N standard thiosulfate and iodine. The end point was observed satisfactorily when a white glass plate was placed below the beaker and at the side opposite the analyst and viewed by reflected light. A second beaker containing water and starch indicator placed beside the titration beaker was of considerable assistance in determining when the end point was reached. It appears that the reaction between selenious acid and thiosulfate is rapid and that standard iodine solution can be added almost immediately.

Table 5 indicates the type of results obtainable when the volumetric procedure with starch, described later, is used. Measured amounts of standard selenium solutions were titrated as unknowns. With 0.01 N standard solutions this procedure is capable of measuring selenium in the range 0 to 2000 gamma, with an average error of approximately 3 gamma. With 0.001 N solutions the procedure is capable of measuring selenium over the range 0–200 gamma, with an average error of approximately 0.5 gamma. With 0.0005 N solutions in the range 0–10 gamma the average error is about 0.2 gamma.

These deviations (Table 5) appear to be of about the same order of magnitude as that in last year's report with the electrometric apparatus. The data indicate that with a given concentration of reagents and with a buret of specified size, the deviation over a definite range is fairly constant. The percentage error of the determination is inversely proportional to the amount of selenium measured. The strength of the titrating solutions to be used is judged by the amount of selenium present for measurement.

This can be estimated roughly from the appearance of the selenium precipitate on the filter pad. When the error under a given set of conditions becomes significant, more dilute standard titrating solutions are used. The data here presented give a good idea of what may reasonably be expected of the method.

SIMPLIFIED PROCEDURES OF SAMPLE PREPARATION AND VOLUMETRIC DETERMINATION

SAMPLE PREPARATION

Place 5–10 grams (dry weight) of the sample in a 600 cc. Pyrex beaker or a Kjeldahl flask, add 0.5 gram of HgO and a cooled mixture of 50 cc. of H_2SO_4 and a volume of HNO_3 equal to 10 cc. per gram of sample taken. Mix thoroughly and allow to stand 30 minutes. Heat *gently* until NO_2 fumes are no longer evolved and the solution turns to a dark brown or SO_3 fumes appear. Cool, and distil with $HBr+Br_2$. The Hg can best be added in solution in HNO_3.

VOLUMETRIC DETERMINATION WITH STARCH INDICATOR

Estimate the amount of the precipitated Se on the filter. (This estimate is used in determining the quantity of $Na_2S_2O_3$ solution to be added later in the titration.) Dissolve the Se in 1–2 ml. of 48% HBr containing 1% by volume of Br_2, using a few drops to rinse the precipitation flask. Wash with a minimum quantity of water so as to keep the volume of filtrate below 20 ml. at most and at about 10 ml. for amounts of Se of 20 gamma and under. Transfer the filtrate and washings to a 30 or 50 ml. beaker. Prepare a few standards containing amounts of Se in the general range of the samples, and 2–3 blanks. Dilute standards and blanks to about the volume of the samples and add the same volume of $HBr+Br_2$ as in the samples. To samples, standards, and blanks add a strong solution of H_2SO_3 until the Br_2 color nearly disappears. (In case all the bromine is reduced, add $HBr+Br_2$ dropwise until the color reappears.) Decolorize with 1–2 drops of 5% aqueous phenol. (It is desirable to reduce the color to a light yellow since tribromophenol is precipitated with excess bromine. The presence of the precipitate, while undesirable, does not ruin the determination.)

Use a stirrer and a 10 ml. buret provided with an extension to dip into the solution being titrated, with the tip so constricted as to make possible the addition of the solution in 0.01–0.02 ml. portions. Place the solution being titrated on a white surface with a white background and view by reflected light.

To the decolorized solution in the 30–50 ml. beaker add about 1 ml. of freshly prepared starch solution. Then add rapidly from a buret a moderate excess of 0.01, 0.001, or 0.0005 N $Na_2S_2O_3$, using the estimate of the precipitated Se as a guide. (1 cc. of 0.01 N $Na_2S_2O_3$ is roughly equivalent to 200 gamma of Se; 1 cc. of 0.001 N to 20 gamma; 1 cc. 0.0005 N to 10 gamma.)

Add about 2 ml. more than the estimated equivalent of $Na_2S_2O_3$ and so select the normality as to keep the volume added between 2 and 10 ml. After about 20–30 seconds add rapidly from a buret a solution of I_2 (approximately the same strength as the $Na_2S_2O_3$ used) until a permanent blue color appears. If less than 1 ml. of I_2 has been added, add 2 ml. more of $Na_2S_2O_3$ and then I_2 until at least 1 ml. is needed to give the blue color. Then add slowly from the dipping buret $Na_2S_2O_3$ of the same strength as before until the color is the same as a blank containing water and 1 ml. of starch solution.

CALCULATIONS

Add up the total volumes of I_2 and $Na_2S_2O_3$ for each determination.

Blanks.—Divide the volume of $Na_2S_2O_3$ by the volume of I_2 to get the factor for conversion of the volumes of I_2 to the equivalent volumes of $Na_2S_2O_3$. Average the results.

Standards.—Multiply the volumes of I_2 by the $I_2-Na_2S_2O_3$ conversion factor, and subtract the product from the total volume of $Na_2S_2O_3$. Divide this number into the quantity of Se in the standard to get the gamma Se/ml. $Na_2S_2O_3$. Average the results.

Samples.—Calculate the volume of $Na_2S_2O_3$ used in the reduction of the Se as directed under "Standards" and multiply by the gamma Se/ml. $Na_2S_2O_3$ value to get the total quantity of Se in the sample in gammas. Divide the total quantity by the weight of the sample in grams taken to get the p.p.m. of Se.

SUMMARY

1. A comparative study made of a number of digestion procedures showed that several, though not all, are equally satisfactory. A simplified HNO_3-H_2SO_4-HgO digestion procedure was developed.

2. The use of the starch indicator in the volumetric determination of selenium by reduction of selenious acid with thiosulfate was studied and found to be quite satisfactory for amounts of selenium above 5 gamma.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods of the Associate Referee and his associates be subjected to further collaborative study, with a view to their tentative adoption by the Association.

(2) That the study of methods of selenium determination be continued.

REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. Hartmann (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

No reports were received on the subjects of Soluble Solids and Effect of Acids on Sugar on Drying and Polariscopic Methods for Jams, Jellies, and Preserves.

Regarding the subject of electrometric titration of acids, Associate Referee Bonnar has been planning the determination of the individual acids comprising a mixture of organic acids titrimetrically by the glass electrode. He has laid the ground work for such an investigation and hopes to have something concrete to report next year.

No collaborative work on fruit acids was undertaken this year. The

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 71 (1938).

Referee has devoted the available time to the study of possible means of simplifying some of the methods now appearing in the *Methods of Analysis.* From the experimental work done, it is believed that it is possible to shorten materially the procedures for the determination of levo and inactive malic acids. A method for the determination of isocitric acid is also indicated. It is intended to continue this work during the coming year.

It is recommended that the various assignments be continued during the coming year.

No report on soluble solids and effect of acids on sugars on drying was given by the associate referee.

REPORT ON ELECTROMETRIC TITRATION OF ACIDITY

By Robert U. Bonnar (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

During the past year the Associate Referee began investigations leading up to determining the amounts of organic acids in a given mixture from the shape of the titration curve. It is possible to reduce this problem to a matrix solution with previously tabulated multipliers. The practical part of the problem is to determine what degree of precision in measurement is necessary to make such a method readily available. The Associate Referee proposes collaborative work to determine what may be expected of commercial hydrogen-ion apparatus after the titration curves of known mixtures of acids have been obtained.

For report on fruit acids see report of the Referee on Fruits and Fruit Products.

No report on polariscopic methods for jams, jellies, and preserves was given by the associate referee.

REPORT ON VITAMINS

By E. M. Nelson (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

It seems desirable to mention some of the developments in the work on vitamins that have occurred during the past year so that efforts toward

the formulation and improvement of methods of assay can be directed toward those control and research problems that give promise of yielding early results. In the field of research two achievements have been conspicuous. Reference is made to the separation of vitamin A in crystalline form and the effectiveness of nicotinic acid in the treatment of blacktongue in dogs.

At ordinary temperatures vitamin A is a viscous liquid but the crystals can be separated from solvents at low temperatures. The preparation of vitamin A in crystalline form will lead to definite progress in its determination spectrographically. There is at present poor agreement with respect to the extinction coefficient of vitamin A, and also in regard to a factor for converting extinction coefficient values to units of vitamin A.

Few pure preparations of vitamin A have been made, and the great instability of the compound has probably not been appreciated. Consequently the data on both biological assays and physical measurements are inadequate. In studies on the crystalline material it has been found that the extinction coefficient of solutions kept at room temperature drops rapidly, but that solutions freshly prepared from material kept at the temperature of solid carbon dioxide have an *E* value of 2100. Biological determinations indicate a potency of 3,000,000 units of vitamin A per gram. Since pure beta carotene by definition has 1⅔ million units per gram, it is interesting to note that the biological process of conversion of beta carotene to vitamin A by the rat is only approximately 50 per cent effective.

Pellagra in man and blacktongue in dogs have for several years been considered to be analogous diseases due to a vitamin deficiency—the so-called "pellagra-preventive factor." Recent studies, however, indicate that pellagra is due to a deficiency of two or more specific factors. There is also evidence that the factor that is curative of a nutritional dermatosis in chicks is resident in the same fraction of the vitamin B complex as are the pellagra and blacktongue preventive factors. The observation that nicotinic acid is effective in the cure of blacktongue in dogs should permit a more accurate determination of the relationships between these nutritional disorders in three different species.

There appears to be no decline in the expansion of the vitamin industry. Cod liver oil imports increased from over 4,500,000 gallons in 1935 to nearly 6,000,000 gallons in 1936, approximately 28 per cent, which increase appears to be reflected largely in the use of this product in poultry feeding. There has also been a marked demand for other fish oils for this purpose. More than 10 per cent of the total farm income is from poultry, and there is a definite place for the use of vitamin D in this industry. There also appears to have been a steady increase in the sale of vitamin D milk, and at the present time there are approximatly 700 dairies delivering some type of this product. It is therefore obvious that vitamin

D assay for cod liver oil and similar products intended for poultry feeding and for the assay of vitamin D milk should receive consideration.

In the pharmaceutical trade a large number of new types of vitamin preparations have apppeared. Although no accurate data are available on the volume of sales, it has been stated that it exceeds that of any other one type of drug preparation. The demand for complex preparations consisting of a mixture of vitamin concentrates and fish liver oils of high vitamin potency has been especially noticeable. Synthetic preparations of vitamin B_1 and vitamin D made by the irradiation of 7 dehydrocholesterol have made their appearance, but their use is apparently limited largely to clinical investigations.

The term "vitamin F" has been used in various ways in scientific literature, but more recently to exploit commercial preparations of linoleic and linolenic acids. Such a designation has no scientific standing, although it is true that it was first used by certain investigators in referring to fatty acids or accompanying substances that are necessary for normal metabolism in the rat. These investigators have requested the Committee on Vitamin Nomenclature of the American Society of Biological Chemists to give no recognition to the use of the term "vitamin F," and they have also expressed their unqualified disapproval of the claims made for it in present-day advertising. Linoleic and linolenic acids can be determined by chemical methods. There appears to be no need for any biological methods for the determination of these substances or for attaching an air of mystery to them by applying the term "vitamin." Furthermore, in the light of results of animal experimentation and of current dietary practices it is hardly conceivable that important fat deficiencies can occur.

Considerable activity in the development of methods of determining vitamins is apparent outside this organization. Some of the investigations will be dealt with specifically in the reports of the associate referees. Spectrophotometric determination of vitamin A, the determination of vitamin B_1 by the rat-curative and bradycardia methods, the chemical determination of vitamin C, the assay of vitamin D milk, and the assay of vitamin D with chicks have all been subjected to more or less extensive investigations. At the present time a large number of laboratories are collaborating in a study of the relative merits of several methods that have been proposed for the assay of vitamin B_1. This work is being done to select the best available method for the U. S. Pharmacopoeia.

The following recommendations[1] are submitted:

(1) That the resignation of C. A. Elvehjem, Associate Referee on Biological Methods for Vitamin B Complex, be accepted.

(2) That O. L. Kline, U. S. Food and Drug Administration, be appointed Associate Referee for vitamin B_1.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 64 (1938).

(3) That the resignation of L. L. Lachat, Associate Referee on Technic and Details of Biological Methods, Vitamin D Carriers, be accepted.

(4) That an associate referee be appointed to succeed Dr. Lachat.

The Referee approves the following recommendations of the associate referees:

(1) *Vitamin A, Mr. Wilkie.*—That collaborative work be done to establish the accuracy and reliability of spectrophotometric equipment by means of a stable inorganic solution absorbing in the region of 328 mu.

(2) *Vitamin B_1, Dr. Elvehjem.*—That the proposed method for the determination of vitamin B_1 in feeding stuffs be given further consideration with a view to its adoption as a tentative method.

(3) *Assay of Vitamin D Milk, Dr. Russell.*—That the tentative method be revised as indicated and that further collaborative studies be made.

(4) *Biological Methods for Assay of Vitamin D Carriers, Mr. Griem.*—That experimental and collaborative work be continued.

REPORT ON VITAMIN A

By J. B. Wilkie (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year certain facts relating to vitamin A determinations were stated and recommendations were made concerning the use of the Vitameter, *This Journal,* **20,** 208 (1937). Since then more data bearing upon the reliability and use of both the Vitameter and the spectrophotometer have become available from several sources. Perhaps the most extensive accumulation of data pertinent to this subject was that obtained under the direction of A. D. Holmes, as Chairman of the Vitamin Assay Committee of the American Drug Manufacturers Association. This study involved 14 laboratories and 34 different samples of fish liver oil.[1]

Variations as high as 100 per cent between maximum and minimum Vitameter E values were reported. A statistical study revealed average positive deviations from the mean as high as 25 per cent and negative deviations as low as −15 per cent. However, each laboratory was found in general to be somewhat more consistent within itself. This fact led the committee to recommend a correction factor for each laboratory in addition to the general factor of 1875 arrived at from a mean of all results. Undoubtedly this correction factor would improve the results for a time, but there is no assurance that an individual factor would remain constant. In fact, as has been pointed out previously by the Associate Referee and others, the Vitameter may quite inadvertently be subject

[1] *J. Am. Pharm. Assoc.,* 26, 525–540 (1937).—The other members of the Committee are A. Black C. R. Eckler, A. D. Emmett, F. W. Heyl, C. Nielsen, and E. G. Quinn.

to change, which obviously would render such a factor inaccurate. Furthermore, as was also pointed out at this meeting last year, the use of a fixed factor (and this holds for a series of fixed factors) to multiply the E value by, is fallacious unless dilution values when plotted against E values can be shown to be a straight line. It has been pointed out in earlier reports that this may not always be the case. Even though a straight line is obtainable, Vitameter characteristics may change, and the Associate Referee is convinced that the most accurate determination of vitamin A with a Vitameter involves the use of Vitameter scale values arbitrarily plotted against U.S.P. cod liver oil concentrations followed by the application of the simple formula given in last year's report:

$$\left.\frac{\begin{array}{c}\text{Actual A units/ml. (as obtained from U.S.P.}\\ \text{Ref. Oil—Vitameter Scale Curve)}\times 100\end{array}}{\text{Concentration of sample in per cent}}\right\} = \begin{array}{l}\text{Vitamin A units/gram}\\ \text{(in unknown sample).}\end{array}$$

This laboratory was one of those cooperating with the American Drug Manufacturers Association, and the results reported were obtained with the Vitameter equipped with the S-1 lamp in place of the recommended copper arc. Results of such a substitution were discussed in the Associate Referee's report last year. Table 2, given in the American Drug Manufacturers' report, shows quite definitely that the apparent E values (the Associate Referee never considered or reported them as E values) are roughly only one-fourth of the values obtained with the conventional apparatus and thus are grossly out of line with other reported Vitameter values. However, if the modified Vitameter values are multiplied by 4 the results fall between the maximum and the minimum values reported from other cooperating laboratories except in two cases, one of which was obviously too much in error for consideration. The results in general so calculated were as close to the average results as those from the other cooperating laboratories listed in the table. This should not be taken to mean that it would be satisfactory to derive an E value from this modified instrument by first multiplying by factor 4, but it does indicate that the particular modified Vitameter used is about as accurate in determining vitamin potency as are those not so modified, and furthermore may even be more accurate when used as it has been customarily used, that is by calculating units per gram directly from the standard cod liver oil independently of the E value.

The Drug Manufacturers Association also reported E values obtained by spectrophotometry. These were lower and generally more consistent than those obtained with the Vitameters.

Another important collaborative investigation concerning the proper conversion factor for changing extinction coefficients to International units per gram, has been reported by E. M. Hume,[1] Secretary of the Vitamin Subcommittee of the Accessory Food Factors Committee ap-

[1] *Nature*, 139, 467 (1937).

pointed by the Lister Institute and Medical Research Council. This investigation was largely instigated by reports throwing doubt upon the validity of the factor 1600 for converting $E^{1\%}_{1\,\mathrm{cm}}$ to International units of vitamin A, as recommended at the 1934 International Vitamin Conference. Values as low as 1000 and as high as 2000 had at various times been recommended.

Ten different laboratories in England and in the United States collaborated with Hume. Feeding oils were prepared from a mixed halibut liver oil, from a concentrate of this oil, and from the beta carotene International standard. All oils, and remains of such oils after the biological tests, were examined spectrophotometrically by R. A. Morton and J. R. Edisbury of Liverpool University. Low factors were obtained from the concentrates. Spectrophotometric results indicated instability of these concentrates as the cause of the low values.

The average factor for the oils not concentrates was found to be 1470. On this basis and in the light of the expected biological variance the evidence was insufficient to warrant a change in the previously recommended factor of 1600. This investigation was limited to one oil and its concentrates. The possible effect of oils from various sources upon the conversion factor will be studied later.

On the basis of other biological assays made both in England and in the United States, in which the potency of the oil is compared with that of the beta carotene International standard, the U.S.P. Reference Cod Liver Oil has been assigned a value of 3000 units per gram.

Of special interest are the E values reported for the U.S.P. Reference Cod Liver Oil which have been accumulated from various laboratories making use of a true spectrophotometer. These values are given in the following table:

	1936 DRUG MFRS. ASSOC.	1936 BARTHEN & LEONARD, WHITE LABS.	1937 ASSOCIATE REFEREE	1936 U S P VITAMIN COMMITTEE CONFERENCE, TAYLOR, ATLANTIC COAST FISHERIES	1936 U S.P. VITAMIN COMMITTEE CONFERENCE MISS COWARD
Untreated Oil	1.46	1.43	1.4	—	—
Treatment unknown	—	—	—	1.4	1.4
Nonsaponified	1.22	1.30	1.0	—	—

The checks indicated in this table are striking when compared with similarly obtained Vitameter results and when the widely varying conditions of obtaining them are considered, but they indicate that a satisfactory degree of precision may be obtained with spectrophotometric methods. However, these figures should not be regarded with too much certainty, because in arriving at them deviations from the mean as large as 19 per cent were noted, in the case of the results reported by the Amer-

ican Drug Manufacturers, and the Associate Referee has only limited data. Also earlier work reported by Morgan in 1935 indicated the *E* value of this oil to be 1.58.[1]

The differences noted in this laboratory between the whole reference cod liver oil and the unsaponified portion may indicate that the previously recommended saponification treatment is either too severe or that some material causing irrelevant absorption is not being removed with the treatments used in other laboratories. There has been no opportunity to investigate this question. For checking such discrepancies and perhaps for use as an alternative method for the vitamin A determination the selective destruction of vitamin A by ultraviolet irradiation may be of value. This method has been used and recommended independently by two foreign investigators, Notevarp[2] and De.[3]

Numerous discrepancies noted in spectrophotometric values obtained with the same material appear to justify the Associate Referee in recommending that the spectrophotometric instruments to be used in vitamin A collaborative work be checked with a stable duplicable inorganic solution having an ultraviolet absorption in the region of 328 mu. It should then be possible to correct the instrument or the technic or to apply a correction factor.

During the past year a method for the preparation of crystalline pure vitamin A was formulated by H. N. Holmes and Ruth Corbet of Oberlin College. In collaborative investigations of the physical properties of this compound *E* values ranging from less than 1600 to 2100 were obtained. One collaborator observed a rapid deterioration of the vitamin A solution at room temperatures, but was able to obtain values of 2100 if such deterioration was avoided. Further investigations of the pure compound should establish the correct *E* value for vitamin A in the near future.

Hickman[4] has described a special vacuum still and a method for preparing vitamin A concentrates that appear to be of importance in preparing such concentrates commercially. His smaller still appears to be a valuable tool in the investigation of any oils containing vitamin A or its esters.

Work in England by Pritchard, Wilkinson, Edisbury, and Morton[5] shows that biologically active material present in fish liver oils can be separated into two portions possessing different types of ultraviolet absorption, but that only one of such portions has the characteristic 328 mu absorption. The other portion apparently has the same absorption but no 328 mu peak. It may have various other peaks, which may or may not be connected with the biological activity. These questions demand consideration in further studies of the vitamin A determination.

[1] *Biochem. J.*, 29, 1645 (1935)
[2] *Ibid.*, 1227–35.
[3] *Indian J. Med. Research*, 24, 3 (1937)
[4] *Ind. Eng. Chem.*, 29, 968–975, 1107–1111 (1937).
[5] *Biochem. J.*, 31, 258 (1937)

No commercial spectrophotometric equipment seemed adequate to meet the needs of the Food and Drug Administration for making routine ultraviolet determinations and carrying on such investigations as have been suggested in this report. Accordingly, equipment for this purpose was devised and constructed. The essential unit of this equipment, the monochromator, was not received until this summer, but the assembly is now usable with combined features of speed, accuracy, and relative freedom from some of the difficulties that have hampered many spectrophotometric systems. An increased flexibility as well as greater precision remains to be accomplished. These matters will receive consideration as promptly as possible. An extended discussion of the details of a spectrophotometer appears to be out of place in this report, but their importance to the work can hardly be overestimated.

New simplified and apparently approved apparatus for the vitamin A determination is gradually appearing. Special reference in this connection may be made to the photoelectric method of McFarlan, Reddie, and Merrill[1] for obtaining practically pure monochromatic light at 3303 Ångstrom units. It may have a more extended application.

To summarize, it is concluded that additional work is desirable along the following lines:

1. Improvement and standardization of optical and electrical equipment especially suited to the need.
2. Collaborative work to establish the accuracy and reliability of spectrophotometric equipment by means of a stable inorganic solution absorbing in the region of 328 mu.
3. Investigation of crystalline vitamin A to determine its correct *E* value, which will lead to a better correlation of all methods for the determination of vitamin A.
4. Investigation of the destruction of vitamin A as a basis for its determination.
5. Investigation of the biological activity of separable fractions of fish liver oil relative to their respective spectrophotometric characteristics.

In view of the extensive investigations of this problem in this country and abroad at the present time and in view of the fact that the spectrophotometric determination of vitamin A will be considered by the International Conference this coming year, further collaborative studies, except as noted in paragraph 2 above, are not recommended at this time, because unnecessary duplication of work should be avoided.

REPORT ON VITAMIN D

By Walter C. Russell (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

It is recommended that further collaborative studies on the development of a method for the assay of vitamin D in milk be undertaken as

[1] *Ind. Eng. Chem.*, 29, 324 (1937).

soon as assayers have had an opportunity to gain experience with the several options which appear in *This Journal* **21,** 90 (1938) and to report their preferences.

REPORT ON CANNED FOODS

By V. B. Bonney (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

During the past year a method for the determination of alcohol-insoluble material in canned peas was submitted to collaborators. This method is given in S. R. A., F. D. 4, Rev. 4, and is adapted from the one suggested by Kertesz, *Food Industries,* **6,** 168 (1934). The method was published in *This Journal,* **21,** 89 (1938).

The sample consisted of two lots of canned peas, designated A and B in the tabulation. Each lot was taken from a single case in the warehouse stock of a commercial canner. Three cans from each lot were submitted to six different groups of collaborators with the request that each can be examined by three analysts in each laboratory. The results of the collaborators are given in the tables.

As was anticipated, the spread between results on different cans from the same lot was large enough to preclude the possibility of considering them as duplicates for purposes of studying the analytical method itself. The figures on the same can by three different analysts (each running check analyses in most cases) afford, however, a judgment of the analytical procedure itself, independent of variations from can to can in the same lot of peas. On the basis of these results it is apparent that the adoption of the method as official (first action) is justified.

The Referee on Tomato Juice made no report, but recommends that the method for determination of chlorides in tomato juice, as given in *This Journal,* **20,** 78 (1937), be made official, first action. On the basis of the collaborative report in *This Journal,* **20,** 217 (1937), concurrence is given to this recommendation.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method suggested for determining alcohol-insoluble material in canned peas be adopted as official (first action).

(2) That the method suggested for determination of chlorides in tomato juice be made official (first action).

(3) That studies of methods of analysis of tomato juice be continued.

(4) That studies of methods for quality factors and fill-of-container be continued.

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[1] For report of Subcommittee C and action of the Association, see *This Journal,* **21,** 68 (1938).

Collaborative results
(Per cent alcohol-insoluble solids)

CAN NO.	ANALYST 1		ANALYST 2		ANALYST 3	
			Baltimore			
A 1	19.03	19.33	19.41	19.19	19.69	19.43
A 2	19.63	19.58	19.54	19.28	19.74	19.58
A 3	18.73	18.90	19.13	19.09	18.87	18.86
B 1	24.63	24.73	25.09	25.02	24.89	24.89
B 2	24.40	24.63	24.92	24.88	24.74	24.68
B 3	25.20	25.08	25.40	25.47	25.16	25.29
			Philadelphia			
A 4	19.09		19.21		19.18	
A 5	20.10		20.08		20.11	
A 6	19.26		19.42		19 30	
B 4	25.05		25.22		25.16	
B 5	24.98		25.18		24.80	
B 6	25.11		25.33		25.31	
			Cincinnati			
A 7	19.64	19.51	19.86	19.80	19.55	19 57
A 8	19.16	19.17	19.33	19.39	19.11	19.24
A 9	18.93	18.90	18.85	18.85	19.02	19.06
B 7	25.18	25.19	25.05	25.04	25 37	25.30
B 8	25.47	25.43	25.23	25.18	25.47	25 57
B 9	25.34	25.34	25.27	25.21	25.37	25 39
			San Francisco			
A 10	19.77	19 59	19.86	19.90	19.94	19 95
A 11	19.69	19.72	19.72	19.93	19.87	19.92
A 12	19.84	19.92	20.09	19.94	20.14	19.89
B 10	24.91	25.06	25.11	25.17	24 97	24.85
B 11	25.12	25.11	25.09	25.11	25 01	25.08
B 12	24.96	24.91	25.30	25.13	25.23	25.19
			Washington, D. C.			
A 13	19.79	19.94	19.72	19.89	19.87	19.81
A 14	19.98	19.86	19.78	19 85	19.97	19.94
A 15	19.64	19.67	19.64	19.69		
B 13	24.86	24.76	24.66	24.79	24 88	24.70
B 14	25.42	25.36	25.30	25.28	25.58	25.41
B 15	25.49	25.31	25.07	25.19		
			Seattle			
A 16	19.40		19.56		19.49	
A 17	20.14		20.13		20.27	
A 18	19.21		19.39		19.22	
B 16	25.07		25.25		25.15	
B 17	24.98		25.10		24.79	
B 18	24.74		24.87		24.72	

No report on tomato products was given by the associate referee.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (The University of Tennessee, Knoxville, Tenn.), *Referee*

During the past year the Referee has obtained collaborative assistance in an exploratory study of the factors that affect the determination of fluoride content of soil. Efforts were directed toward circumventing the interference attributable, in particular, to aluminum and phosphates. This work was independent of the studies that have been conducted by the Associate Referee for the Less Abundant Elements.

The several reports of the associates carry recommendations with which the Referee concurs.

REPORT ON HYDROGEN-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS

By W. T. McGeorge (The University of Arizona, Tucson, Ariz.), *Associate Referee*

In making a *p*H determination of alkaline soils the value obtained varies with the soil-water ratio, and the selection of this ratio is more or less arbitrary. If a curve is drawn by plotting soil-water ratio against *p*H value, the change between the ratios of 1:1 and 1:10 is rather sharp. Above 1:10 there is little or no change with dilution. Therefore it can be assumed that the 1:10 ratio will represent the maximum potential *p*H of the soil.

Since this *p*H value is rarely if ever reached under field conditions, it seems that the *p*H of the soil at field moisture content should be the most useful value.

During the past year the adaptation of the spear-type glass electrode to *p*H determinations at low soil-moisture contents was studied. This type of electrode is made sufficiently rugged to permit its being pressed into the soil at very low moisture content. The results being obtained with it look promising, and readings have been obtained as low as 6 per cent moisture. The values obtained at these low moisture contents appear to be accurate and can be closely duplicated, as shown by the results already obtained on many replicate determinations on the same soil.

It is planned to solicit the cooperation of a number of workers at the western experiment stations in order to learn just how generally this method can be applied to alkali soils.

It is believed that this spear-type electrode is a piece of equipment that should be of great value to agronomists and plant physiologists and to others interested in the relation between *p*H, plant growth, and natural plant coverage.[1]

REPORT ON HYDROGEN-ION CONCENTRATION OF SOILS OF HUMID REGIONS

A STUDY OF THE FACTORS THAT INFLUENCE THE *p*H VALUE OF COASTAL PLAIN SOILS AND METHODS FOR DETERMINATION

By JACKSON B. HESTER (Virginia Truck Experiment Station, Norfolk, Va.), *Associate Referee*

Since the introduction of soil acidity as a factor in crop production, and since methods for determining the reaction of soils have been developed, perhaps no other field of research has been of more practical value to the grower on Coastal Plain soils. Previous to 1919 most fertilizer materials were either neutral or slightly acid-forming in the soil. The introduction of acid-forming materials into the fertilizer mixtures has greatly increased the value of tests for soil acidity. Since the hydrogen-ion concentration of solutions and suspensions is easily and rapidly determined, and since the *p*H value of a soil is a measure of the intensity of the acidity, a knowledge of the *p*H value of the soil becomes a practical asset to the grower.

TABLE 1.—*The pH values of Coastal Plain soils analyzed at Norfolk*

pH VALUES	JULY 1935–JUNE 1936		JULY 1936–JUNE 1937	
	NUMBER	PER CENT	NUMBER	PER CENT
–4.4	27	0.6	42	0.7
4.5–4.9	459	9.8	548	9.3
5.0–5.4	1229	26.4	1896	32.4
5.5–5.9	1609	34.6	1847	31.6
6.0–6.4	917	19.7	1060	18.0
6.5–6.9	318	6.9	337	5.7
7.0–7.4	70	1.5	106	1.8
7.5–	19	0.4	37	0.6

Soil acidity is a product of weathering. Acid soils are found only in regions where drainage exceeds evaporation. Because of relatively high rainfall a majority of the soils on the Atlantic Seaboard are extremely acid. In fact, the data given in Table 1 show that approximately 40 per cent of the soils tested at the Station in 1936 and 1937 were more acid than *p*H 5.5. The data further show that only a comparatively small

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 63 (1938).

number of the soils tested above pH 7.5. Thus, in consideration of soil tests for the pH value of Coastal Plain soils one is mainly concerned with methods for acid conditions.

COLLOIDAL ACID STRENGTH OF THE SOIL

When suitable methods for determining the pH value of the soil are selected the acids involved in the soil should be considered. In the process of weathering of soils hydrated silicates of iron and aluminum are developed. These amphoteric products[1] show an acid reaction when the strong replaceable bases (calcium, magnesium, potassium, and sodium) are removed by leaching. The degree to which the strong bases are replaced with hydrogen from the exchange complex represents the percentage saturation of the clay. As a result of vegetation the decomposition of organic debris develops humic matter. Humus behaves similarly to the silicates in regard to acid reaction. Soil humus has a strong buffer capacity. The influence that the clay and humic acids exert upon the pH value and the exchange capacity of the soil is shown in Table 2.

TABLE 2.—*Buffer capacity of H-Clay and H-Humus*

SOIL TYPE	pH VALUE			EXCHANGE CAPACITY		
	COLLOID	CLAY	HUMUS	COLLOID	CLAY	HUMUS
					m.e./gram	
Norfolk	4.7	5.1	3.6	0.28	0.22	1.45
Bladen	3.6	3.9	3.5	0.35	0.32	1.25
Portsmouth	3.5	3.8	3.2	0.58	0.28	1.70

The colloidal material was isolated from the Norfolk, Bladen, and Portsmouth soils by shaking, sedimentation, and filtration. The colloids were then electrodialyzed in a Mattson cell and the various experiments conducted. For the analysis of the clay the organic matter was destroyed with 30 per cent hydrogen peroxide and redialyzed. The exchange capacity was determined by leaching with a neutral salt solution.

These data show that a fundamental difference exists in the acidities of the various clays. The clay content of the Norfolk soil is least acid in character and that of the Portsmouth is the most acid. It further shows that the humate fraction is more acid than the clay fraction. Undoubtedly the two materials are united in the soil and exert an influence upon each other. This fact is brought out in the exchange capacity of the humus that was isolated from the soil.

The fact that humus from different sources has different residual acidity strengths is shown in Table 3. To explain further certain of the data, humus isolated from Dismal Swamp peat was treated with dif-

[1] Hester and Shelton, Va. Truck. Expt. Sta. Bull. 84 (1934).

TABLE 3.—*Buffer capacity of humus from different sources*

SOURCE	pH H-HUMUS	EXCHANGE CAPACITY
		m.e./gram
Manure	3.5	1.67
Dismal Swamp Peat	3.0	2.95

ferent quantities of iron from ferric chloride (Table 4). The exchange capacity of the humus was decreased by the ampholyte. Thus, iron and aluminum act as bases with silicates and humates but are not easily replaced with neutral salts. Iron and aluminum decrease the residual acidity of the soil and thus soils with a high iron and aluminum content and low humus content possess a low residual acid strength.

TABLE 4.—*pH value of electrodialyzed humus from Dismal Swamp peat with different ratios of Fe_2O_3*

MOLECULAR RATIO			EXCHANGE CAPACITY	pH VALUE
HUMATE	Fe_2O_3	M_2O		
			m.e./gram	
1	—	0.67	2 95	3.0
1	0.75	0.61	1.88	3.3
1	1.50	0.53	1.67	3.5

The measurement of the *p*H value of the soil gives an indication of the percentage saturation of the residual acid strength of the soils with strong bases.

SOLUBLE ACID CONTENT

In addition to the colloidal acids of the soil soluble acids[1] are involved. As a result of biological oxidation of organic matter in the soil, soluble acids (nitric, hydrochloric, and sulfuric) are formed. The extent to which these acids occur in the soil varies. Immediately after heavy rainfall the nitric, sulfuric, and hydrochloric acid content may be negative. However, after a long period when leaching does not occur, nitrates, sulfates, and chlorides may accumulate to a considerable extent (Table 5). These data likewise show that a variation of as much as 0.8 of a *p*H unit may exist in the soil due to soluble acids. Furthermore, a method for measuring the *p*H value of the soil will be influenced by these acids.

*p*H METHODS COMPARED

The glass electrode, quinhydrone, and colorimetric methods are the most promising procedures[2] for determining the *p*H of Coastal Plain soils. The glass electrode represents a high initial cost and is delicate to operate.

[1] Hester and Shelton, *Am. Soc. Agron.*, 25, 299 (1933); Va. Truck Expt. Sta. Bull. 94 (1937).
[2] Snyder, E. F., U. S. Dept. Agr. Circ. 56 (1935).

It is perhaps the most accurate method yet proposed for the pH value of the soil.

The quinhydrone method has certain obvious faults. Soluble manganese interferes with its use in soils, although the Associate Referee found interference from manganese in soils around Norfolk to be rare. As the soil reaction approaches pH 7.0 it becomes difficult to determine the correct reading due to the potentiometer having a tendency to drift. The quinhydrone procedure with the calomel cell and salt bridge is extremely rapid and easy to operate.

TABLE 5.—*Influence of mineral acids in the soil (from biological activity) upon the pH value of the soil*

(Sassafras sandy loam)

MONTH	pH OF SOIL	PARTS PER MILLION OF LEACHABLE ACIDS HNO_3	H_2SO_4	HCl	pH OF SOIL LEACHED*
December	5.14	76	122	1.3	5.48
July	4.67	156	147	1.9	5.44

* Leached until leachable acid remaining was only a trace.

The third method that is being compared in this study is a colorimetric method recommended by Hester and Shelton.[1] It consists of precipitating the clay with an insoluble salt, barium sulfate, and determining the pH value of the liquid by means of certain dyes. Its low cost and ease of handling make it a good method when a limited number of tests are to be made.

TABLE 6.—*Comparison of different methods for the pH value of a Norfolk sandy loam*

COLORIMETRIC	QUINHYDRONE	GLASS ELECTRODE	P.P. 2 MILLION REPLACEABLE CaO
4.5	4.56	4.60	476
4.6	4.71	4.72	588
5.1	5.17	5.32	840
5.75	5.57	5.85	1302
6.0	5.90	6.07	1582
6.35	6.13	6.40	1620
6.85	6.71	7.10	1778

The comparison of the three methods on certain soil types is shown in Tables 6 and 7. These data indicate that under average conditions the three methods compare favorably. The Associate Referee made none of the given pH readings. The soil samples shown in Table 7 were sent to various collaborators,* and their results were used. The object of this

[1] Va. Truck Expt. Sta. Bull. 95 (1935).

* T. F. Bridgers, Wilson, N. C.; C. R. Byers, Carteret, N. J.; F. B. Carpenter, Richmond, Va.; D. M. Goss, New Brunswick, N. J.; E. W. Magruder, Norfolk, Va.; J. M. Newbold, Fayetteville, N. C.; C. L. Paul, Charleston, S. C.

Table 7.—*Correlation of the different methods on two general soil types*

COLORIMETRIC	QUINHYDRONE	GLASS ELECTRODE
	Elkton fine sandy loam	
5.15	5.20	5.20
5.25	5.29	5.29
5.05	5.28	5.10
5.15	5.28	5.25
	Sassafras sandy loam	
4.9	4.90	4.92
4.9	4.90	4.94
4.4	4.35	4.35

procedure was to determne the accuracy of the methods in the hands of various operators rather than in the hands of a single operator.

SOIL AND WATER RATIO

Many questions have been asked the Associate Referee about the proportion of soil to water to use in the *p*H test. In the work described above an approximately 1 to 2 soil-water proportion was used. The previous discussion shows that the organic matter and clay content is responsible for the *p*H value of the soil. The clay and organic matter content of every soil differs. Thus, to get a constant ratio of colloid and water, it would have to be determined on each soil and a different amount of soil taken for each soil sample. Fortunately for rapidity of operation, the buffer capacity of the clay and ionization of the hydrogen ion take care of this situation. Therefore, a ratio of approximately 1 to 2 of soil and water has been found highly satisfactory.

CONCLUSIONS

For Coastal Plain soils there are three good methods available for determining the *p*H value of the soil. The glass electrode has a wide range of adaptability and is the most accurate method of the three. It unfortunately has a high initial cost. The quinhydrone procedure is satisfactory for most Coastal Plain soils. It is subject to drift at high *p*H values and in the presence of manganese. The colorimetric method has a wide range of adaptability, is accurate, and has a low initial cost. The colorimetric method is not an absolute method, however.

From the number of samples of soil that the Experiment Station has been called upon to handle, it is estimated that the demand for the test by the growers in the future will be very great. Further, it has been known that many commercial organizations are offering this service to the growers. When the number of tests to be made is small it is not profitable to purchase expensive apparatus.

REPORT ON LIMING MATERIALS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

The present method for the analysis of liming materials gives the potential neutralizing capacity of the total content of calcium and magnesium present as either oxides, hydroxides, carbonates, or silicates. The factor of proportions of limestone separates does not enter into the determination, since the charges are completely dissolved. The degree of fineness materially affects the speed with which limestone and dolomite undergo disintegration in soil and in fertilizer mixtures. The influence of fineness upon the speed of limestone and dolomite decomposition in soils has been studied [1,2,4,5] but the disintegration in soils will vary with soil type, flora, season, and depth of incorporation.

It seems important to have some rapid chemical method for the evaluation of limestones and dolomites of different finenesses. The Associate Referee has studied the utility of the ammonium chloride distillation procedure[3] for this objective. The results appear promising and it is therefore recommended[6] that a study be made of the adaptability of the boiling ammonium chloride method for the evaluation of rate of availability of ground limestones and dolomites.

DETERMINATION OF ABSORBED BASES AND EXCHANGE CAPACITY OF SOILS BY THE BOILING AMMONIUM CHLORIDE PROCEDURE

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

The writer, as Associate Referee on Liming Materials, undertook a collaborative study of the ammonium chloride procedure for the determination of absorbed calcium and magnesium and exchange capacity of soils, as recommended at the 1936 meeting of the Association, to establish the precision and accuracy of the determination of absorbed bases by the proposed method in comparison with other methods. To simplify the work for the present, the soils selected were principally calcium-saturated and contained varying amounts of calcic limestone. It is particularly essential to establish the ease and accuracy of determining the absorbed calcium in the presence of large supplies of calcium carbonate.

The following collaborators participated in the present study: A. P.

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[1] MacIntire and Shaw, *Soil Science*, 20, 403 (1925).
[2] *Ibid.*, *J. Am. Soc. Agron.*, 22, 272 (1930).
[3] Shaw and MacIntire, *Soil Science*, 39, 359 (1935).
[4] Taylor and Pierre, *J. Am. Soc. Agron.*, 27, 764 (1935).
[5] White, J. W., Pa. Agr. Exp. Sta. Bull. 149 (1917).
[6] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 63 (1938).

Thomas, Maryland; A. L. Prince and S. J. Toth, New Jersey; A. T. Perkins, Kansas; C. E. Allard, Rhode Island; and Brooks Robinson, Tennessee.

INSTRUCTIONS TO COLLABORATORS ON BASE EXCHANGE VALUES OF SOILS

The object of the study presented here is to establish the accuracy and expediency of the boiling ammonium chloride procedure for the determination of (a) base exchange capacity and (b) absorbed calcium, magnesium, potassium, and sodium in one extraction. Advantages claimed for this procedure are greater accuracy in the determination of absorbed calcium and magnesium in heavily limed soils and the general expediency when the several bases are to be determined. Calcium and magnesium are dissolved completely by the ammonium chloride extraction. It is therefore necessary to determine the carbonate content of the soil, and to correct for either calcium when calcic carbonate alone is present or for both calcium and magnesium when dolomite is present. The samples used for the present study contain only carbonate of calcium. To simplify the immediate problem, and because of the possible diversity of the carbonate carbon dioxide procedure, these values, determined at the Tennessee Agricultural Experiment Station, are submitted for use by the collaborators. It is suggested that the results be reported in the following manner.

A.O.A.C. sample No.	Available Ca	Available Mg	Carbonates Ca	Carbonates Mg	Absorbed Ca	Absorbed Mg	Exchange capacity
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*

The principal determinations obtained by this procedure should be paralleled by determinations obtained by some other procedure preferred by the individual collaborator. It will be helpful to include in the report any digressions from the usual technic along with comments as to the advantages of the alternative procedures used.

DETERMINATION OF EXCHANGE CAPACITY AND ABSORBED BASES IN SOILS BY THE BOILING AMMONIUM CHLORIDE PROCEDURE

Grind soils to pass a 60-mesh sieve and mix by rolling. Weigh a 10 gram charge of the air-dried sample into a 600 ml. beaker. Add 100 ml. of normal NH_4Cl solution prepared from the highest grade c.p. salt. Add 200 ml. of distilled water, place coverglass over beaker, and heat to boiling by means of a Bunsen burner. Boil gently at first; then increase to vigorous boiling until the volume is reduced to about 100 ml. At this stage apply the bromocresol green test for NH_4OH in the escaping steam. (Completeness of the digestion is indicated by failure of the vapor to change the yellow spot on the test paper to a distinct blue.) Continue adding hot water in 200 ml. portions and boil until a negative test is obtained. For the preparation of the test solution and other details of the test, see *Soil Science*, 39, 372. Remove the beaker from source of heat, cool, add 15 ml. of 0.1 *N* NH_4OH, stir, and let stand

about 30 minutes. Filter on a 56 mm. Büchner filter, taking care to obtain a clear filtrate. Transfer all soil by policing beaker and cover-glass, using normal NH_4Cl solution neutralized with ammonia to pH 7.0 by phenol red. Continue to wash with neutralized NH_4Cl to a volume of 250 ml. The soil residue is used for determination of exchange capacity, and the filtrate is saved for subsequent determination of extracted calcium and magnesium, and if desired, also sodium and potassium.

Exchange capacity.—Transfer the soil through a short-stemmed funnel into an Erlenmeyer flask with a minimal quantity of distilled water, using a policeman. Add about 100 ml. of ethyl alcohol, stopper the flask, and shake vigorously. Pour contents onto a prepared 56 mm. water-wetted filter, and wash with small portions of alcohol to a volume of about 500 ml. Should slow filtration develop, return filter to flask, introduce filter paper pulp, and agitate to a uniform suspension to facilitate filtration and washing; wash edge of filter to prevent crust formation. Transfer the alcohol-washed residue to a distillation flask; add an aqueous suspension of MgO and distil about 200 ml. into 0.1 N acid. On the basis of a 10 gram charge, each ml. of 0.1 N NH_4OH distilled represents 1 m.e. per 100 grams of soil.

Exchangeable Ca and Mg.—Precipitate Ca as oxalate directly in the clear 250 ml. solution. Remove Mn from filtrate before determination of Mg by evaporating the Ca filtrate to a small volume and decompose NH_4 salts by the addition of 15–20 ml. of HNO_3 in covered beakers and gently boiling to dryness. Digest the residue for a few minutes on a hot plate with 10 ml. of HCl (1+1); dilute to about 25 ml., heat over a Bunsen burner, neutralize with ammonia, and precipitate Mn by addition of a small lump of $(NH_4)_2S_2O_8$, maintaining a slightly alkaline condition by frequent additions of a few drops of NH_4OH (1+1) to a faint odor while digesting. On coagulation of the hydrated oxide, filter and wash with 2% hot neutral NH_4Cl to a volume of about 60 ml. Precipitate the Mg in the filtrate in the usual manner.

RESULTS

Because of lack of time or of misunderstanding, the collaborators confined their work to determinations by the boiling ammonium chloride procedure. One collaborator reported results obtained by the use of the ammonium acetate procedure, but these were not adequate for comparative purposes, since the attack by the acetate upon carbonate is partial, whereas the boiling ammonium chloride effects complete carbonate decomposition. Collaborative results are detailed in Tables 1, 2, and 3, and summarized in Table 4.

Calcium extraction results by the ammonium chloride procedure (Table 1) represent calcium in both absorbed state and carbonate form. Considering the quantities involved, a fair agreement for Samples 1, 2, 4, and 6 was obtained by the several collaborators. For soils Nos. 3 and 5 fair concordance was secured by three of the five collaborators. These results are considered indicative of the possibility of precision with increasing familiarity with the technic of this procedure.

The absorbed magnesium results (Table 2) appear rather discordant. This may be readily explained by the difficulty in effecting complete removal of the manganese, and also possibly by the presence of alumina that escaped precipitation. Careful determinations of the magnesium extracted from several soils by the ammonium chloride and ammonium

acetate procedures show that the acetate gives inadequate removals for samples of known magnesium absorptions. Correctness in the determination of the magnesium content of the extracts needs careful study.

The ammonia absorption, or base exchange capacity, results are given in Table 3. Contrary to the more general practice, the alcohol wash of the ammonium chloride treated soils was not neutralized. Accordingly, it may be expected that the results should be somewhat lower than those obtained by use of ammonia-neutralized alcohol washings. The results, however, show fair concordance. The use of neutralized alcohol at this Station has given values from 2 to 4 m.e. higher than those shown in Table 3. These results are incorporated in Table 4. Neutralization of the alcohol with ammonia is not a simple process. Excessive amounts of ammonia are required to bring the alcohol to pH 7.0, and this corrected alcohol easily and quickly acquires lower pH upon the least exposure to the atmosphere. Better agreement for ammonia-absorption values and absorbed Ca+Mg values is obtained for residues washed with neutralized alcohol, as indicated by comparisons of the first and third columns from the right of Table 4. Further research should determine

TABLE 1.—*Exchangeable calcium by the boiling ammonium chloride procedure—milliequivalents per 100 grams of air-dried soil*

SOIL NO.	COLLABORATORS*					
	1	2	3	4	5	AVERAGE
	m.e.	*m e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1	13 5	13.7	13.5	13.7	13 2	13.5
2	11.6	11.4	11.8	11.8	15.3	11.7
3	92.1	77.9	78.0	80.6	87.8	83 3
4	44.7	45.1	45.2	46.4	—	45 4
5	34.1	36.7	35.8	38 7	41.3	37.3
6	8.4	8.5	8.3	9.9	10.6	9.1

* The numbers assigned to the collaborators are not in the order given in the text.

TABLE 2.—*Exchangeable magnesium by the boiling ammonium chloride procedure*

SOIL NO.	COLLABORATORS					
	1	2	3	4	5	AVERAGE
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m e.*	*m e*
1	3.2	1.3	1.3	3.4	4 6	2.8
2	1 2	1 6	1 5	1 9	1.4	1 5
3	5.5	3.8	4.4	5.0	5.0	4.7
4	0.9	1.0	1 0	1.5	—	1.1
5	3.8	2.4	2.3	6.6	5 2	4.1
6	2.0	1.1	1.4	2.4	3.1	2 0

whether the "ammonia-neutralized" alcohol has the effect of an additional ammoniacal treatment.

The results for absorbed calcium and magnesium are given in Table 4. These values were obtained by adding the means of the calcium and magnesium results of Tables 1 and 2 and subtracting therefrom the respective carbonate carbon dioxide values. This absorbed calcium and magnesium may be completely or partly in the exchangeable form. The

TABLE 3.—*Exchange capacity—absorbed ammonia by the boiling ammonium chloride procedure*

SOIL NO.	COLLABORATORS					
	1	2	3	4	5	AVERAGE
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1	8.9	8.2	8.8	8.9	7.3	8.4
2	8.2	7.1	8.1	8.3	6.4	7.6
3	8.7	8.3	8.7	9.1	8.1	8.6
4	9.6	9.8	10.5	10.3	—	10.1
5	26.6	25.9	26.2	28.4	24.2	26.3
6	6.1	5.9	6.0	6.5	5.9	6.1

TABLE 4.—*Relationship between the absorbed calcium and magnesium and the absorption capacity, as determined by the boiling ammonium chloride procedure*

SOIL NO.	EXTRACTED BY BOILING NH_4Cl SOLUTION			CARBONATE CO_2	ABSORBED Ca+Mg AVERAGES	ABSORBED NH_3	
	Ca	Mg	Ca+Mg			UNNEUTRALIZED ALCOHOL, AV.	NEUTRALIZED ALCOHOL
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1	13.5	2.8	16.3	1.6	14.7	8.4	11.6
2	11.7	1.5	13.2	0.8	12.4	7.6	11.8
3	83.3	4.7	88.0	59.6	28.4	8.6	12.1
4	45.4	1.1	46.5	33.6	12.9	10.1	12.4
5	37.3	4.1	41.4	3.9	37.5	26.3	33.3
6	9.1	2.0	11.1	1.8	9.3	6.1	8.0

extent of its exchangeability will depend on the manner in which the exchange reaction is carried out, as discussed above. It is strongly indicated, however, that a large part of the absorbed calcium of the heavily limed soil, No. 3, is in a non-exchangeable form, although readily dissolved by ammonium chloride.

RECOMMENDATIONS[1]

It is recommended—

(1) That the boiling ammonium chloride procedure be studied further, with the objective of increasing speed of extraction.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 63 (1938).

(2) That both the absorbed calcium and magnesium values be compared with those obtained by other methods with soils of known history.

(3) That a study be made as to degree of optimal ammoniation of the alcohol wash as affecting the determination of exchange capacity.

REPORT ON LESS COMMON METALS IN SOILS

By J. S. McHargue (Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*

As soon as the information of Mr. Winter's death was received a telegram was sent to E. J. Miller, Mr. Winter's assistant, stating that some work had been done during the past year to ascertain the fluorine content of normal plant tissues. The method used is the one published by Hoskins and Farris[1] of the University of California.

However, the results thus far obtained are not satisfactory. Accordingly this subject will be studied further during the next year, and no formal report of the results obtained will be given at this time. The fusion method suggested by the Associate Referee a few years ago for the determination of fluorine in soils is quite tedious and time-consuming, but thus far no shorter and more satisfactory procedure has been devised.

No report on selenium in soils was given by the associate referee.

REPORT ON FERTILIZERS

By G. S. Fraps (Agricultural Experiment Station, College Station, Texas), *Referee*

The task of the fertilizer chemist increases in complexity from year to year. Recent amendments to the North Carolina fertilizer law permit (but do not require) the minimum calcium oxide (Ca) to be claimed in all mixed fertilizers, maximum sulfur in tobacco fertilizers, and also additional plant food, elements, compounds, or classes of compounds determinable by chemical control methods. These rather broad provisions may also be implied in the laws of some of the other states, though not expressly declared. Because of these legal provisions, it seems desirable to have an associate referee on calcium and sulfur in fertilizers and also on copper and zinc. It will probably be necessary only to apply well-known methods, but their application to fertilizers should be studied.

A. J. Cox, of the Department of Agriculture of California, states that they are unable to use the new method for potash in fertilizers because the California law expressly requires that the analysis shall state the per-

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[1] *Ind. Eng. Chem. Anal. Ed.*, 8, 6–9 (1936).

centage of potash soluble in distilled water. It seems desirable that the Associate Referee on Potash should ascertain which other states, if any, are unable to use the new method for potash, on account of similar legal provisions of the law, and recommend what should be done about it.

The recommendations of the associate referees are given in their reports.

The Referee on Fertilizers recommends[1]—

(1) That an associate referee be appointed to study methods for the determination of calcium and sulfur in fertilizers.

(2) That an associate referee on copper and zinc be appointed, and that he be requested to cooperate with the Associate Referee on Secondary Elements, so that the analysis may be made in one solution, if possible.

(3) That the Associate Referee on Potash be requested to ascertain whether there is any other state besides California that can not use the present official method for potash on account of provisions of the laws regarding water-soluble potash, and to make any recommendations regarding this matter that seems desirable.

REPORT ON PHOSPHORIC ACID

CITRATE-INSOLUBLE PHOSPHORIC ACID IN AMMONIATED MIXTURES CONTAINING DOLOMITE

By WILLIAM H. ROSS, *Associate Referee*, L. F. RADER, JR., and K. C. BEESON (Fertilizer Research Division, Bureau of Chemistry and Soils, Washington, D. C.)

In his report at the last meeting of this Association, the Referee on Fertilizers, G. S. Fraps (3), recommended that the Associate Referee on Phosphoric Acid make a study (a) of the influence of different filter papers on the determination of water-soluble P_2O_5; and (b) of the effect of permitting the washed residue to stand for a time before digestion in the ammonium citrate solution. A study of these two phases of phosphoric acid analysis has been made by J. Richard Adams of the Fertilizer Research Division, Bureau of Chemistry and Soils. The results of his work are described in a separate report (1).

A recommendation was also made by the Associate Referee on Phosphoric Acid (13) that a further study be made of the nature of the citrate-insoluble components of phosphate materials with a view to improving the method of determining the availability of such materials.

The ammonium citrate method for determining phosphate availability was developed by Fresenius, Neubauer, and Luck (4) in 1871. This method was adopted by the Association of Official Agricultural Chemists at its first meeting in 1884, and with slight modifications it still remains

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 61 (1938).

the official method for determining phosphate availability. The method is an arbitrary one, however, and while it is recognized as giving satisfactory results in the evaluation of superphosphates, it is not so well adapted to the analysis of certain other phosphates that have recently come on the market. Thus it is known that the method gives a higher availability rating to an ammoniated superphosphate in a mixture of low P_2O_5 content than to the same ammoniated superphosphate in a mixture having a relatively high P_2O_5 content.

In commercial ammoniation, superphosphate is treated with a maximum of about 3 per cent of ammonia, which quantity does not decrease the availability of the superphosphate at ordinary temperatures, but further additions may cause a loss of available P_2O_5 due to formation of tricalcium phosphate (6, 16). The effect of dolomite on an ammoniated superphosphate depends on the temperature at which the mixture is stored. Little or no change occurs in dolomite-containing mixtures that are maintained at normal temperatures (2, 7, 10, 11), but a marked increase in citrate-insoluble P_2O_5 may take place in mixtures of this kind that are heated to temperatures above normal as a result of ammoniation or other treatment (2, 7).

It has been observed that the citrate-insoluble P_2O_5 in certain mixtures decreases with decrease in the size of the sample taken for analysis (14, 15). In mixtures prepared under other conditions, the size of the sample has little effect on the values found for citrate-insoluble P_2O_5. These results might be explained on the assumption that the citrate-insoluble residues in some mixtures consist largely of a material such as tricalcium phosphate, which is somewhat soluble in citrate solution, and that the principal phosphatic component of other residues is a material such as calcium hydroxyphosphate or fluorapatite, which is only slightly soluble in citrate solution.

In compliance with the recommendation adopted last year, a study was undertaken of the composition of the citrate-insoluble phosphates that form during storage of ammoniated mixtures containing dolomite. The study has not been completed, and only a preliminary report can be presented at this time.

EXPERIMENTAL

The initial materials used in this work were monocalcium phosphate, tricalcium phosphate, bone ash, and phosphate rock. The monocalcium phosphate was of analytical reagent grade. The tricalcium phosphate was prepared by neutralizing C. P. lime with the equivalent quantity of C. P. orthophosphoric acid, evaporating to dryness, and igniting to constant weight at 900° C. Analyses of these different phosphatic materials are given in Table 1.

The tricalcium phosphate and bone ash samples were acidulated alone

TABLE 1.—*Composition of phosphatic materials and of dolomite*

SAMPLE NO.	MATERIAL	MOISTURE AND ORGANIC CARBON	CaO	MgO	Na_2O+K_2O	Al_2O_3	Fe_2O_3	SiO_2	CO_2	SO_3	F	P_2O_5
							per cent					
49	Monocalcium phosphate	5.00	19.59	none	none	none	none	none	none	none	none	56.00
3	Tricalcium phosphate	none	53.68	none	none	none	none	none	none	none	none	45.64
10	Bone ash	0.18	54.18	none	0.93	none	none	trace	0.70	0.36	0.05	40.46
14	Florida pebble rock	1.74	49.05	0.25	0.26	1.05	0.70	7.48	1.48	0.20	3.95	35.37
15	Tennessee brown rock	1.42	49.19	0.02	0.45	1.16	2.52	5.92	1.96	0.70	3.81	34.44
7	Nauru Island phosphate	none	52.24	none	none	0.36	0.18	none	none	none	2.42	36.50
31	Dolomite[1]	0.08	29.01	17.10	none	none	0.55	none	41.87	none	none	none

[1] Same sample as used by Beeson and Ross (2).

and when separately mixed with silica, iron phosphate, aluminum phosphate, and calcium fluoride. The resulting products were cured for a month as in the manufacture of ordinary superphosphate. The cured superphosphates were then treated with the same quantity of ammonia per unit of P_2O_5, amounting to 3 per cent on the basis of a 20 per cent superphosphate. Portions of each ammoniated superphosphate and of mixtures of 100 parts of the ammoniated superphosphate with 20 parts of dolomite were adjusted to a moisture content of 5 per cent and stored in bottles at temperatures of 30°, 45°, 60° and 75° C. A rapid loss of moisture from the stored samples was prevented by closing each bottle with a rubber stopper, through which passed a long glass tube having a capillary opening at the top. Samples of the stored mixtures were withdrawn at intervals and analyzed for citrate-insoluble fluorine and P_2O_5.

The results obtained with the ammoniated superphosphates made from tricalcium phosphate and from bone ash are given in Table 2. Both sets of experiments are in agreement in showing (a) that the citrate-insoluble P_2O_5 in a 3 per cent ammoniated superphosphate undergoes a relatively small increase in the absence of fluorine even when stored at 75° C for 180 days; (b) that the citrate-insoluble P_2O_5 in a 6 per cent ammoniated superphosphate increases more rapidly at 75° C. than in a 3 per cent ammoniated sample; (c) that the presence of silica, iron, and aluminum has little or no effect on the results; (d) that the presence of fluorine as a calcium fluoride causes an increase in citrate-insoluble P_2O_5 in ammoniated mixtures stored at 30° C., but not in nonammoniated mixtures even when dolomite is present; (e) that fluorine-containing nonammoniated mixtures, as well as those that are ammoniated, show an increase in citrate-insoluble P_2O_5 when stored at temperatures above 30° C.; (f) that the addition of dolomite to an ammoniated mixture stored at 30° C. causes an increase in citrate-insoluble P_2O_5 when fluorine is present but not when it is absent; (g) that the addition of dolomite to mixtures stored at 75° C. causes a marked increase in citrate-insoluble P_2O_5 in all ammoniated mixtures; and (h) that the effect of dolomite on ammoniated mixtures stored at 75° C. is about the same whether fluorine is or is not present.

The results obtained with the superphosphate made from phosphate rock are given in Table 3. They show that the source of the rock has little effect on the results and that some loss of available P_2O_5 occurs even at temperatures as low as 30° C. At this temperature the presence of dolomite does not cause any increase in citrate-insoluble P_2O_5, but when the mixtures are stored at temperatures above 45° C. those that contain dolomite show higher conversion that those that do not.

The results on citrate-insoluble fluorine and citrate-insoluble P_2O_5 in fluorine-containing mixtures stored at 75° C. are given in Table 4. In these tests the fluorine in the citrate-insoluble residues increased as a

TABLE 2.—*Citrate-insoluble P_2O_5 in ammoniated superphosphate*
(Ammonia content = 3%[a])

SAMPLE NO.	MATERIAL ADDED TO $Ca_3(PO_4)_2$ BEFORE ACIDULATION	WT. OF MATERIAL ADDED PER 100 g.	52.7° Bé H_2SO_4 ADDED PER 100 g.	DOLOMITE ADDED PER 100 g. AMMONIATED SUPER-PHOSPHATE	INITIAL P_2O_5 IN AMMONIATED MIXTURE		INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE AT—					
									75° C. FOR—			
					TOTAL	CITRATE-INSOLUBLE	30° C. FOR 180 DAYS	60° C. FOR 180 DAYS	15 DAYS	30 DAYS	90 DAYS	180 DAYS
		grams	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	Prepared from tricalcium phosphate											
31	None		83.3	none	26.22	1.10	0.06	—	—	0.00	0.02	0.14
32[b]	None		83.3	none	25.77	1.09	0.06	—	—	0.94	2.53	3.05
411	None		83.3	20	22.15	1.35	0.00	0.00	1.57	5.10	8.78	11.82
41	Silica	8.0	83.3	none	25.05	0.34	0.16	—	—	0.03	—	0.16
51	Iron phosphate	4.0	97.0	none	25.94	0.40	0.07	—	—	—	—	0.25
61	Aluminum phosphate	5.0	100.0	none	25.62	0.36	0.00	—	—	—	—	0.34
361	Calcium fluorine	6.6	93.5	none	23.50	0.33	1.95	2.64	4.37	4.67	6.07	6.70
420[c]	Calcium fluorine	6.6	106.7	20	20.20	0.03	0.10	0.42	0.20	0.60	1.55	1.58
421	Calcium fluorine	6.6	106.7	20	20.00	0.75	4.11	7.41	4.13	9.30	10.55	12.23
	Prepared from bone ash											
	Material added to bone ash before acidulation											
371	None		84.7	none	22.73	2.51	0.00	0.29	0.00		0.19	0.61
391	None		84.7	20	18.75	2.45	0.00	3.85	4.80		7.80	9.60
111	Silica	8.0	85.0	none	23.09	3.22	0.88	0.88	—		0.76	1.43
113	Silica	8.0	85.0	20	19.66	3.65	0.00	3.37	—		5.37	5.37
121	Iron phosphate and aluminum phosphate	4.0 5.0	86.7	none	24.53	3.70	0.58	0.38	—		0.43	0.90
123	Iron phosphate and aluminum phosphate	4.0 5.0	86.7	20	20.58	3.65	0.02	0.45	—		6.20	7.10
381	Calcium fluoride	6.6	95.7	none	20.53	0.50	1.31	2.89	3.40		5.15	6.30
400[c]	Calcium fluoride	6.6	95.7	20	17.75	0.40	0.00	0.73	1.27		3.85	5.32
401	Calcium fluoride	6.6	95.7	20	17.53	2.20	5.34	8.38	2.20		8.80	10.24

[a] On basis of superphosphate containing 20% P_2O_5.
[b] Ammoniated to 6.0%.
[c] Not ammoniated.

TABLE 3.—*Citrate-insoluble P_2O_5 in ammoniated superphosphate prepared from phosphate rock*
(Ammonia content = 3%[a])

SAMPLE NO.	SOURCE OF PHOSPHATE	52.7° Bé H_2SO_4 ADDED	DOLOMITE ADDED PER 100 g. AMMONIATED SUPER-PHOSPHATE	INITIAL P_2O_5 IN AMMONIATED MIXTURE		INITIAL CITRATE-INSOLUBLE F	INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE AT—						INCREASE IN CITRATE-INSOLUBLE F AFTER STORAGE AT
				TOTAL	CITRATE-INSOLUBLE		30° C. FOR 180 DAYS	45° C. FOR 180 DAYS	60° C. FOR 180 DAYS	75° C. FOR— 30 DAYS	75° C. FOR— 60 DAYS	75° C. FOR— 180 DAYS	75° C. FOR 180 days
		grams	grams	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
141	Florida pebble	81.5	none	21.83	0.88	0.18	1.65	3.34	3.52	2.31	3.09	5.30	0.37
143	Florida pebble	81.5	20	18.64	0.70	0.17	1.92	3.75	4.40	4.53	6.32	9.72	0.66
161[b]	Florida pebble	—	none	20.66	0.64	0.19	2.16	3.91	3.76	3.94	3.57	4.96	0.17
163[b]	Florida pebble	—	20	17.77	0.38	0.16	1.85	3.72	6.87	3.88	5.49	9.27	0.61
151	Tennessee brown rock	90.6	none	20.21	0.23	0.14	1.42	2.07	2.12	2.29	2.43	5.25	0.16
153	Tennessee brown rock	90.6	20	16.82	0.28	0.11	0.94	3.27	5.27	3.04	5.52	8.62	0.55
171[b]	Tennessee brown rock	—	none	20.16	0.54	0.20	1.71	2.51	2.29	2.31	2.97	3.71	0.16
173[b]	Tennessee brown rock	—	20	17.12	0.30	0.17	1.02	2.93	4.62	3.45	3.76	4.40	0.26
71	Nauru Island phosphate	91.7	none	23.36	0.61	0.08	1.69	2.57	2.57	2.20	3.10	4.09	0.30
73	Nauru Island phosphate	91.7	20	19.48	0.51	0.09	1.49	2.94	4.79	2.91	3.78	7.89	0.71

[a] On basis of superphosphate containing 20 per cent P_2O_5.
[b] Commercial superphosphate.

TABLE 4.—*Citrate-insoluble fluorine and P_2O_5 in ammoniated superphosphates*

SAMPLE NO.	SOURCE OF PHOSPHATE	TOTAL		INITIAL CITRATE-INSOLUBLE		COMPOSITION OF CITRATE-INSOLUBLE RESIDUES AFTER STORAGE AT 75° C. FOR—									
						15 DAYS		30 DAYS		60 DAYS		90 DAYS		180 DAYS	
		F	P_2O_5	F	P_2O_5	F	P_2O_5	F	P_2O_5	F	P_2O_5	F	P_2O_5	F	P_2O_5
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
361	$Ca_3(PO_4)_2+CaF_2$	1.52	23.50	0.60	0.33	0.83	4.70	0.80	5.00	0.88	5.30	0.81	6.40	0.97	7.03
421[a]	$Ca_3(PO_4)_2+CaF_2$	1.40	20.00	0.61	0.75	0.93	4.88	0.96	10.05	0.85	9.70	0.79	11.30	0.97	12.98
420[a,b]	$Ca_3(PO_4)_2+CaF_2$	1.39	20.20	0.28	0.03	0.55	0.23	0.69	0.63	0.48	1.28	0.57	1.55	0.93	1.61
381	Bone ash $+CaF_2$	1.60	20.53	0.05	0.50	0.19	3.90	0.37	6.10	0.49	5.35	0.52	5.65	0.53	6.80
400[a,b]	Bone ash $+CaF_2$	1.40	17.75	0.03	0.40	0.21	1.67	0.29	2.70	0.42	3.78	0.46	4.25	0.61	5.72
401[a]	Bone ash $+CaF_2$	1.38	17.53	0.30	2.20	0.51	4.40	0.66	8.90	0.68	10.40	0.81	11.00	0.95	12.44
141B	Florida pebble rock	2.16	21.32	0.14	1.05	0.28	2.95	0.33	3.00	0.32	4.00	0.32	4.55	0.44	5.10
151B	Tennessee brown rock	2.08	19.12	0.13	0.17	0.10	1.05	0.07	1.85	0.05	2.55	0.08	3.05	0.10	4.12

[a] Contains 20 grams dolomite per 100 grams superphosphate.
[b] Not ammoniated.

rule during the first 15 days of storage and then remained more or less constant with further storage. The citrate-insoluble P_2O_5 on the other hand continued to increase through the entire period of storage. The fluorine in the citrate-insoluble residues from the ammoniated rock superphosphate samples that had been stored at 75° C. for 180 days was not sufficient to account for all the P_2O_5 as fluorapatite. In the case of the Tennessee superphosphate not more than 20 per cent of the citrate-insoluble P_2O_5 could have been present as a fluorine compound.

The results obtained with samples prepared by ammoniating pure monocalcium phosphate and with mixtures of 100 parts of ammoniated

TABLE 5.—*Citrate-insoluble P_2O_5 in ammoniated monocalcium phosphate mixtures* (Ammonia content = 3%[a])

SAMPLE NO.	MATERIAL ADDED TO $Ca(H_2PO_4)_2$ BEFORE AMMONIATION	WT. OF MATERIAL ADDED PER 100 g.	DOLOMITE ADDED PER 100 g. AMMONIATED PRODUCT	INITIAL P_2O_5 IN AMMONIATED MIXTURE		INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE AT—			
				TOTAL	CITRATE-INSOLUBLE	30° C. FOR 15 DAYS	75° C. FOR 1 DAY	75° C. FOR 7 DAYS	75° C. FOR 15 DAYS
		grams	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
451	None	None	None	56.00	None	0.00	0.00	0.10	0.26
461	None	None	20	45.76	None	0.00	0.00	0.10	0.34
471	Calcium fluoride	6.6	None	52.62	0.93	3.50	9.89	10.88	11.16
481	Calcium fluoride	6.6	20	42.78	0 94	1.00	5.09	11.83	12.12

[a] On basis of superphosphate containing 20% P_2O_5.

monocalcium phosphate and 6.6 parts of calcium fluoride are shown in Table 5. These samples in the order given in the table differ from Samples 31, 411, 361 and 421, respectively, of Table 2 in that they do not contain any calcium sulfate, which is normally associated with the monocalcium phosphate in an ordinary superphosphate. A comparison of the results obtained with these two sets of samples, as given in Tables 2 and 5, shows that the presence of fluorine has a marked effect on the citrate-insoluble P_2O_5 after only one day's storage at 75° C., whereas the reversion due to dolomite does not become apparent until after a storage period of about 7 days.

The increase in citrate-insoluble P_2O_5 in the ammoniated monocalcium phosphate samples 471 and 481 amounted, after 15 days' storage at 75° C., to 11.16 and 12.12 per cent of the sample, respectively, or to 21.20 and 28.33 per cent of the total P_2O_5 in the sample. In the corresponding ammoniated superphosphate samples, 361 and 421 (Table 2), the increase in citrate-insoluble P_2O_5 amounted under the same conditions to 18.59 and 20.65 per cent of the total P_2O_5. This indicates that fluorine-containing ammoniated monocalcium phosphate mixtures revert more rapidly on the basis of their total P_2O_5 content than do the corresponding superphos-

phate mixtures, due no doubt to the more intimate contact of the reacting components of the more concentrated mixtures.

DISCUSSION

The principal phosphatic components of a superphosphate or monocalcium phosphate that has been treated with 3 per cent of ammonia are monoammonium phosphate and dicalcium phosphate (6, 16). Dicalcium phosphate undergoes hydrolysis in the presence of water to form first tricalcium phosphate and then calcium hydroxyphosphate, which is stable in contact with water (8, 12).

$$10CaHPO_4+2H_2O=3[Ca_3(PO_4)_2]\cdot Ca(OH)_2+4H_3PO_4 \qquad 1$$

If dolomite is present, it will react at 75° C. with the monoammonium phosphate in the mixture to evolve ammonia, as shown in equation 2 (2).

$$5NH_4H_2PO_4+3CaCO_3\cdot MgCO_3=Ca_3(PO_4)_2+3MgNH_4PO_4+2NH_3+6H_2O+6CO_2 \qquad 2$$

In the presence of ammonia the conversion of dicalcium phosphate to the hydroxyphosphate will then proceed as follows (16):

$$10CaHPO_4+4NH_4OH=3[Ca_3(PO_4)_2]\cdot Ca(OH)_2+4NH_4H_2PO_4+2H_2O \qquad 3$$

Precipitated tricalcium phosphate, calcium hydroxyphosphate, and fluorapatite give the same diffraction pattern, and x-ray analysis, therefore, can not be applied at present in distinguishing between these three compounds. The ratio of P_2O_5 to CaO in tricalcium phosphate is 0.8447, and in calcium hydroxyphosphate or fluorapatite it is 0.7598. It follows, therefore, that these three compounds can be distinguished from one another in a pure state by chemical analysis but not when other compounds of calcium or phosphorus are present. No direct evidence of the nature of the citrate-insoluble compounds formed in this investigation has been obtained, but reactions that are known to take place indicate that the reversion that occurs in fluorine-free, dolomite-containing mixtures stored at 75° C. is due to the formation of calcium hydroxyphosphate.

The presence of fluorine greatly increases the rate of the reversion, particularly in mixtures stored at 30° C. If this is due to the formation of fluorapatite, as represented in Equation 4, the reaction should be accompanied by an increase in water-soluble P_2O_5.

$$9CaHPO_4+CaF_2=3[Ca_3(PO_4)_2]\cdot CaF_2+3H_3PO_4 \qquad 4$$

That an increase in water-soluble P_2O_5 actually takes place, is shown by the data in Table 6. Thus, Sample 361, which contained fluorine but no dolomite, showed a gradual increase in water-soluble P_2O_5 when the mixture was stored at 75° C. from 15 to 180 days. When dolomite was present and fluorine absent (Sample 411), the water-soluble P_2O_5 rapidly decreased

at first and then increased slightly. A similar change occurred when the sample contained both dolomite and calcium fluoride, but the presence of the fluoride caused a more rapid increase in water-soluble P_2O_5 following the initial decrease. These results are in agreement with the reactions

TABLE 6.—*Water-soluble P_2O_5 in ammoniated superphosphate prepared from tricalcium phosphate*

SAMPLE NO.	MATERIAL ADDED TO AMMONIATED SUPERPHOSPHATE	INITIAL WATER-SOLUBLE P_2O_5	WATER-SOLUBLE P_2O_5 AFTER STORAGE AT 75° C. FOR—			
			15 DAYS	30 DAYS	60 DAYS	180 DAYS
		per cent	*per cent*			
361	Calcium fluoride	14.75	15.00	16.00	17.13	17.68
411	Dolomite	10.18	2.25	1.00	—	1.43
421	Dolomite+calcium fluoride	13.20	8.75	2.95	—	4.90

known to take place at temperatures above normal in ammoniated mixtures containing dolomite. As shown in Equation 2, the dolomite first reacts with the monoammonium phosphate in the mixture to decrease the water-soluble P_2O_5, while the evolved ammonia in turn tends to increase the water-soluble P_2O_5 by ammoniation of the dicalcium phosphate present (Equation 3). The second of these two reactions takes place more slowly than does the first (5), and the net result is first a decrease and then an increase in water-soluble P_2O_5.

CONCLUSIONS

Although the work is not complete, the results indicate that the di- and tricalcium phosphates initially formed in the ammoniation of superphosphate undergo hydrolysis in storage at temperatures above normal to form calcium hydroxyphosphate and that the presence of dolomite increases the extent to which these reactions take place. In the presence of fluorine, the reaction apparently proceeds to the formation of a fluophosphate such as fluorapatite, as suggested by MacIntire and his coworkers (9). The proportions of these citrate-insoluble phosphates in an ammoniated superphosphate or a mixture of an ammoniated superphosphate and dolomite depend on the basicity of the mixture, the moisture and fluorine content, the concentration of the reacting components, and the temperature and time of storage.

It is recommended[1] that the work be continued.

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REPORT ON PHOSPHORIC ACID

A. *Effect of the Method of Filtering on the Determination of Water-Soluble P_2O_5*

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Two general procedures have been in use for the determination of water-soluble P_2O_5. In one of these procedures, as represented by the method of Fresenius, Neubauer, and Luck (4), 10 grams of the sample is placed on a filter and gradually extracted with cold water until the wash water no longer gives an acid reaction. The solution is then made up to a liter and mixed. In the other procedure, as represented by the so-called "Dresden method" (10), 20 grams of superphosphate is freed from the greater part of its water-soluble P_2O_5 by stirring with water and decanting and if necessary repeating the operation. The residue is then ground fine, washed into a liter flask, and digested 3 hours in water with frequent shaking. The liquid first poured off is then added, the flask is filled to the mark, and its contents are finally mixed and filtered through a dry filter.

The method adopted by the Association of Official Agricultural Chemists at its first meeting in 1884 consisted for the most part of a combination of these two procedures. The steps in the method were as follows:

> Place 2 grams on a filter, add a little water, let it run out before adding more water, and repeat this treatment cautiously until no phosphate is likely to precipitate in the filter. When the sample is nearly washed in this manner, transfer to a mortar, rub with a rubber-tipped pestle to a homogenous paste, return to the filter and wash with water until the washings no longer react acid with delicate test paper. Mix the washings and take a suitable aliquot for the determination of its P_2O_5 content.

In 1886 the phrase "the washings no longer react acid with delicate test paper" was changed to read "the filtrate measures not less than 250 cc."

(14). Chazal (1) later called attention to the dependence of the quantity of water-soluble P_2O_5 on the size of the filters used during its extraction, and the method was again modified to provide for a 9 cm. filter. In 1890 Frear (3) reported the results of comparative experiments using (a) simple washing on the filter, and (b) washing and rubbing in a beaker. The difference in the results did not seem to justify the longer time required in carrying out the rubbing method. This observation was confirmed by other chemists, and in the following year the official method for water-soluble P_2O_5 was again changed to eliminate all rubbing and to require washing on the filter paper alone. This method with minor changes, such as the substitution of a 1 gram charge for 2 grams (11), has remained the official method until the present time.

In his report of last year the Referee on Fertilizers (2) referred to claims that have been made that the kind of filter paper used affects the results obtained for water-soluble P_2O_5. A recommendation was accordingly adopted that a further study be made of this step in the method. The work described in the first part of this report was undertaken in compliance with this recommendation.

EXPERIMENTAL

The phosphatic materials used in this work were as follows:

1. Ordinary superphosphate made from Tennessee rock, containing 20.06% total P_2O_5.
2. Ammoniated superphosphate, containing 5.62% N and 19.45% total P_2O_5.
3. Mixed fertilizer (4-16-4). This mixture was more than a year old when used in these tests. The formula was as follows:

MATERIAL	MIXED FERTILIZER 4-16-4
	lbs. per ton
Superphosphate, 20.2% P_2O_5	1225
Double superphosphate, 46.7% P_2O_5	157
Ammonia	37
Ammonium sulfate, 20.5% N	146
Sodium nitrate, 16.3% N	123
Kieserite, 31.5% MgO	50
Potassium chloride, 58.0% K_2O	138
Dolomite	124
	2000

The water-soluble P_2O_5 in these samples was determined (a) by washing each sample on six different filter papers under gravity and (b) by washing on one filter paper (Whatman No. 5) under suction. The results obtained are given in Table 1. Each result represents the mean of closely agreeing duplicate determinations, replicated three times, or six determinations in all.

TABLE 1.—*Effect of kind of filter paper used in the determination of water-soluble P_2O_5*

FILTER PAPER	SUPERPHOSPHATE			AMMONIATED SUPERPHOSPHATE			MIXED FERTILIZER (4-16-4)		
	TIME OF FILTERING RANGE IN 6 DETNS.	TIME OF FILTERING MEAN	WATER-SOLUBLE P_2O_5	TIME OF FILTERING RANGE IN 6 DETNS.	TIME OF FILTERING MEAN	WATER-SOLUBLE P_2O_5	TIME OF FILTERING RANGE IN 6 DETNS.	TIME OF FILTERING MEAN	WATER-SOLUBLE P_2O_5
	minutes	*minutes*	*per cent*	*minutes*	*minutes*	*per cent*	*minutes*	*minutes*	*per cent*
Whatman No. 5, with suction	11	11	12.12	8–9	9	3.36	8–9	8	7.14
Whatman No. 12, folded	39–45	42	12.47	29–43	37	3.74	21–29	25	7.73
Whatman No. 40	49–61	53	12.48	35–60	47	3.79	20–30	25	7.77
Whatman No. 2	50–60	54	12.61	39–63	50	3.75	22–38	33	7.78
S and S No. 606	61–92	74	12.71	62–85	72	3.75	32–122	71	7.87
Whatman No. 42	75–97	82	12.60	55–89	74	3.76	48–67	58	7.90
Whatman No. 5	88–109	101	12.83	64–80	73	3.79	35–77	64	7.86

Table 1 shows that when the washing was done under gravity, the different kinds of filter paper used in the tests had little or no effect on the results obtained for water-soluble P_2O_5, and that washing under suction gave values that were significantly lower than those obtained by washing under gravity.

CONCLUSIONS

The kind of filter paper on which the sample is washed in the determination of water-soluble P_2O_5 has little if any effect on the results, provided the washing is done under gravity. Washing by suction gives lower results than does washing under gravity.

B. Variation in Citrate-Insoluble P_2O_5 with the Time Interval Between the Water Extraction and Citrate Digestion.

The official method for determining citrate-insoluble P_2O_5 in acidulated goods directs that after the sample is washed, as in the determination of water-soluble P_2O_5, the residue and filter paper be dropped into the ammonium citrate solution at a temperature of 65° C. However, the method does not state whether the citrate digestion should be made soon after the sample is washed or whether the washed residue may be allowed to stand overnight or longer.

Although it has been claimed that a delay in the digestion of the washed residue usually has little effect on the results, individual cases have occurred in which a continuous reversion of the P_2O_5 takes place on prolonged standing of the washed residue (10). A recommendation was accordingly adopted at the last meeting of the A.O.A.C. that a further study be made of the maximum permissible interval between the washing of the sample and the digestion of the residue in citrate solution (2).

EXPERIMENTAL

The materials used in these tests were the same as those described in the first part of this report. Three sets of samples of each material were washed as directed in the official method for water-soluble P_2O_5. A Whatman No. 40 filter under gravity was used. One set of the washed residues was digested in citrate solution at once, and the other two sets at intervals of 17 and 96 hours. The funnels in which the filters containing

TABLE 2.—*Variation in citrate-insoluble P_2O_5 with the time interval between the washing and citrate digestion*

TIME INTERVAL	CITRATE-INSOLUBLE PHOSPHORIC ACID (P_2O_5) SUPERPHOSPHATE	AMMONIATED SUPERPHOSPHATE	MIXED FERTILIZER (4-16-4)
hours	*per cent*	*per cent*	*per cent*
0	0.12	6.00	0.22
17	0.12	7.52	0.30
96	0.10	7.66	0.41

the washed residues were allowed to stand before digestion were covered with watch-glasses, and the tips were kept under water to prevent drying of the residues. The results obtained are given in Table 2.

DISCUSSION

Table 2 shows that the citrate-insoluble P_2O_5 in the washed residues from the ammoniated superphosphate and mixed fertilizer samples increased on standing before digestion, while the washed residue from the superphosphate sample showed no increase. A superphosphate in which the ratio of free orthophosphoric acid to moisture present is greater than 0.22 contains water-soluble monocalcium phosphate but little or no di- or tricalcium phosphates (5). The superphosphate (Sample 1) used in the tests had a moisture content of 2.2 per cent and a free acid content of 1.5 per cent. The ratio of free acid to moisture in the sample was therefore 0.68, or more than sufficient to prevent hydrolysis of the monocalcium phosphate present. Numerous tests have also shown that a monocalcium phosphate containing this ratio of free acid to moisture does not undergo hydrolysis when washed as directed in the official method for determining water-soluble P_2O_5. It may, therefore, be concluded that the water-insoluble residue from Sample 1 contained little or no calcium phosphate other than that represented by the undecomposed rock in the sample. A superphosphate, on the other hand, that has been treated with upwards of 3 per cent of ammonia (Sample 2) contains monoammonium phosphate and dicalcium phosphate, or di- and tricalcium phosphates, but little or no monocalcium phosphate (6, 13). It follows, therefore, that the washed residues of the ammoniated superphosphate and the ammoniated mixed fertilizer samples used in the tests contained dicalcium phosphate, while the washed residue from the superphosphate sample contained little or no dicalcium phosphate. It is known that mono-, di- and tricalcium phosphates undergo hydrolysis to form calcium hydroxyphosphate (7,9). This compound is not decomposed by water and its solubility in neutral ammonium citrate solution is less than half as great as that of tricalcium phosphate (11). It might seem, therefore, that on standing the reduced citrate solubility of the P_2O_5 in the washed residues from the ammoniated superphosphate and mixed fertilizer samples is due to a partial transformation of di- or tricalcium phosphate present into calcium hydroxyphosphate. The preceding report by Ross, Rader, and Beeson (12) shows, however, that while calcium hydroxyphosphate is formed in fluorine-free ammoniated mixtures that are stored for a time at temperatures above normal, no loss of available P_2O_5 occurs in mixtures of this kind when stored at 30° C. The situation is different when fluorine is present. A marked increase in citrate-insoluble P_2O_5 then occurs in a relatively short time even at temperatures as low as 30° C., due apparently to the formation of fluorapatite, as suggested by MacIntire and his co-workers (8), or

other fluophosphate. All three samples used in the tests contained fluorine. It is therefore to be expected, as was found by experiment, (a) that prolonged standing of the washed residue from the ammoniated superphosphate and mixed fertilizer samples would cause an increase in citrate-insoluble P_2O_5 by conversion of the dicalcium phosphate or di- and tricalcium phosphates present into a fluophosphate of lower solubility; and (b) that the washed residue from the superphosphate sample would undergo little or no increase in citrate-insoluble P_2O_5 due to its limited content of either di- or tricalcium phosphate.

CONCLUSIONS

The citrate-insoluble P_2O_5 in materials such as ammoniated superphosphate, which contain di- or tricalcium phosphate, increases with prolonged standing of the washed residue before digestion in citrate solution, but this factor has little effect on the citrate-insoluble P_2O_5 in materials such as ordinary superphosphate, which do not contain appreciable quantities of either di- or tricalcium phosphate.

It is recommended[1] that the work be continued by collaborative study.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, **21**, 61 (1938).

REPORT ON NITROGEN*

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

Further collaborative work was carried on this year on the determination of water-insoluble nitrogen in cyanamid. Two years ago a method was proposed and adopted as official (first action), *This Journal*, **19**, 284 (1936). The essential part of this method consisted of grinding the sample in water prior to thorough washing on the filter paper, as well as the use of a specific grade and size of filter paper. The results from 16 collaborators indicated that this method was the best of the five procedures tried out.

This year the collaborative work was repeated, and the proposed method was compared with two other modifications tried out in 1935. The detailed instructions sent out to the collaborators were as follows:

INSTRUCTIONS TO COLLABORATORS

Two samples of cyanamid will be submitted: granular and pulverized. The sample of granular cyanamid should be ground as rapidly as possible in a mortar and transferred to the bottle.

In the following experiments, proper washing of the cyanamid on the filter paper is important. Be sure to allow complete drainage before adding more water to the filter paper. Use Whatman No. 2, 11 cm. filter paper for all filtrations.

EXPERIMENTS

Series I.—Run each sample in triplicate according to the method adopted as official, first action, in 1935, *Methods of Analysis, A.O.A.C.*, p. 27, par. **36**.

Series II.—Add 2 grams of ground rock phosphate to a 2 gram sample of cyanamid, mix thoroughly, transfer to the filter, and wash to 350 cc. From this point continue as directed in Series I. Run in triplicate.

Series III.—Weigh out a 2 gram sample and transfer to the filter paper; wash with about 7 cc. of ethyl alcohol, and then with 350 cc. of distilled water. Determine the nitrogen in the residue as usual.

The collaborators were the following:

(1) C. W. Hughes, Agr. Exp. Station, Lafayette, Ind.
(2) E. F. Boyce, Agr. Exp. Station, Burlington, Vt.
(3) E. K. Rist, Bureau of Chemistry and Soils, Washington, D. C.
(4) L. J. Hardin, Agr. Exp. Station, Knoxville, Tenn.
(5) R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
(6) H. C. Batton, Swift and Company, Baltimore, Md.
(7) A. H. Allen, Virginia-Carolina Chemical Corp., Richmond, Va.
(8) R. C. Koch, Swift and Company, Hammond, Ind.
(9) W. L. Adams, Agr. Exp. Station, Kingston, R. I.

The data from the collaborative study for both granular and pulverized cyanamid are compiled in Table 1. Under Series I are given the data for

* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

the proposed official method. It will be noted that the results under this series are far more concordant among the different collaborators than are the results under Series II and III.

It is true that occasionally a collaborator obtains comparable results with Series I using the modifications of Series II and III, but in general the results obtained for water-insoluble nitrogen under Series II and III are higher and far more irregular than those from Series I. With the granular cyanamid, the average water-insoluble nitrogen found by the proposed method (Series I) was 0.71 per cent as compared with 0.89 per cent and 0.81 per cent from Series II and III, respectively. With the pulverized cyanamid, the average water-insoluble nitrogen under Series I was 0.82 per cent as compared to 0.97 per cent and 0.99 per cent from Series II and III. These collaborative results are similar to those obtained two years ago. The slight tendency for the pulverized cyanamid to run a little higher in water-insoluble nitrogen may probably be attributed to the difficulty of completely moistening this finely divided material and its subsequent reaction with water. As pointed out in the report given in 1935, *This Journal*, **19**, 284 (1936), the essential point in connection with the analysis of cyanamid for water-insoluble nitrogen is the complete reaction of the cyanamid with water (not merely a superficial washing on the filter paper). The proposed method makes possible such a reaction, and fairly concordant results have been obtained.

TABLE 1.—*Results of analysis of granular and pulverized cyanamid for water-insoluble nitrogen (each result represents an average of three determinations)*

COLLABORATORS	GRANULAR CYANAMID			PULVERIZED CYANAMID		
	SERIES I	SERIES II	SERIES III	SERIES I	SERIES II	SERIES III
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.59	0.72	0.69	0.66	0.76	0.73
2	0.63	0.69	0.72	0.81	0.91	0.80
3	0.65	0.76	0.72	0.76	0.77	1.61
4	0.89	1.35	1.20	1.40	1.71	1.39
5	0.53	0.53	0.54	0.56	0.52	0.56
6	0.78	1.33	0.90	0.77	1.06	1.00
7	0.67	0.96	0.81	0.78	1.14	1.12
8	0.97	1.13	1.18	0.92	1.07	0.94
9	0.65	0.58	0.53	0.75	0.75	0.75
Average	0.71	0.89	0.81	0.82	0.97	0.99

According to a suggestion made by the General Referee on Fertilizers, several different lots of reduced iron obtained from different chemical companies were examined for nitrogen. Five gram samples were used for analysis and were digested with sulfuric acid, as in the usual procedure for total nitrogen. No nitrogen was found in the fresh samples. One sample,

which had been in the laboratory for about 2 years, contained minute traces of nitrogen. However, since the Devarda method has been made official for the determination of nitrates both in mixed fertilizers and nitrate salts, it seems unnecessary to retain the reduced iron method. The latter method requires two stages in its operation, while the Devarda method is carried out by a simple distillation process. In order to eliminate unnecessary methods, the Associate Referee suggests that the reduced iron method for the determination of nitrate nitrogen in fertilizers be withdrawn. However, a statement should be appended to the Devarda method indicating that in the analysis of nitrate salts an aliquot of the nitrate solution equivalent to 0.5 gram of the sample should be used.

It was also suggested by the General Referee on Fertilizers that the Associate Referee on Nitrogen cooperate with the Referee on Standard Solutions in improving, if necessary, the methods for volumetric solutions used in the estimation of nitrogen. During the past year the Associate Referee on Nitrogen has served as a collaborator with the Referee on Standard Solutions. Approximately 0.1 *N* hydrochloric acid and sodium hydroxide solutions submitted by the Referee on Standard Solutions were standardized according to the directions given in *Methods of Analysis A.O.A.C.*, 1935, pages 681–682. In this method acid potassium phthalate is used as the primary standard. In addition to this work, the Associate Referee compared the above method of standardization with the silver chloride method given under the fertilizer section of *Methods of Analysis, A.O.A.C.*, 1935, page 23. Although the Referee on Standard Solutions will report in detail his collaborative findings, a brief summary of the results of this Associate Referee on the comparison of the two methods is given as follows:

Comparison of methods of standardizing solutions of HCl and NaOH
(Results represent averages of 4 determinations)

SOLUTION APPROXIMATELY 0.1 *N*	NORMALITY BY PHTHALATE METHOD	NORMALITY BY AgCl METHOD
NaOH	0.09953	0.09939
HCl	0.09965	0.09951

It would appear from these results that there is a good agreement between the two methods. It is often desirable to standardize the acid prior to the alkali by means of a direct gravimetric procedure. In fact, this is necessary in laboratories that use ammonium hydroxide as a base in their nitrogen work. Also, since it is not always convenient for the fertilizer chemist to obtain acid potassium phthalate of the necessary purity, it would seem wise to retain the silver chloride method for standardization of hydrochloric acid as given on page 23 of *Methods of Analysis*.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method adopted two years ago as official (first action) for the determination of water-insoluble nitrogen in cyanamid be adopted as official (final action).

(2) That the reduced iron method for the determination of nitrate nitrogen in mixed fertilizers or nitrate salts described under section **31**, page 26 of the 1935 edition of *Methods of Analysis*, be eliminated (first action).

(3) That the following paragraph be added to the Devarda method, section **33**, pages 26–27, 1935 edition of *Methods of Analysis*:

> In the analysis of nitrate salts proceed as directed above but use 25 cc. of the nitrate solution equivalent to 0.50 gram of the sample.

(4) That the Associate Referee cooperate further with the Referee on Standard Solutions in improving methods for preparing volumetric solutions used in the estimation of nitrogen.

REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS*

By JOHN B. SMITH, *Associate Referee*, and E. J. DESZYCK (Agricultural Experiment Station, Kingston, R. I.)

The magnesium studies reported in 1936, *This Journal*, **20**, 252 (1937), were continued, both in this laboratory and with the generous assistance of several collaborators, and a beginning was made in the comparison of methods for manganese.

MAGNESIA

In arranging the report, experimental conditions are described and the data presented, then follows an attempt to show the bearing of the results on the different fractions of magnesia that have been suggested as important.

COLLABORATIVE ANALYSES

The samples distributed (Table 1) were made in this Laboratory and are intended to represent the various types of fertilizer formulas. Samples 1 and 2 are bases, 4–13.5–0, cured for 30 days at 60° C. and described in the previous report. Sample 1 was kieserite; Sample 2, a very reactive, 80–100-mesh magnesium limestone; Samples 3 and 4 were dry mixtures containing a commercial manganous sulfate as well as magnesium. Sample

[1] For report of Subcommittee A and action of the Association, see *This Journal*, **21**, 61 (1938)
* Contribution No. 524 of this Station.

TABLE 1.—*Samples for collaborative analysis*

SAMPLE*	INGREDIENTS	POUNDS	ADDED MgO (PER CENT) FROM KIESERITE	FROM Mg LIMESTONE	TOTAL
1. 4–13.5–0	Superphosphate	1456	1.41		1.41
	Urea ammonia liquor	235			
	Sulfate of ammonia	96			
	Kieserite	109			
	Sand	104			
2. Same as 1, but instead of kieserite,					
	Mg limestone 12 A (18%MgO)	150		1.43	1.43
	Sand	63			
3. 7–6–6	Superphosphate	353	1.00	1.00	2.00
	Diammonium phosphate	112			
	Cottonseed meal	200			
	Sulfate of ammonia	405			
	Nitrate of soda	125			
	Muriate of potash	240			
	Mg limestone (18% MgO)	110			
	Kieserite (29.65% MgO)	67			
	$MnSO_4$ (24% Mn)	85			
	Sand	303			
4. 5–9–8	Superphosphate	1060	1.48	0.77	2.25
	KNO_3 C. P.	344			
	Nitrate of soda	325			
	$MnSO_4$ (24% Mn)	85			
	Kieserite (29.65% MgO)	100			
	Mg limestone (18% MgO)	86			
5. 5–8–7	4–13.5–0 Base	908	2.61	0.65	3.26
	Cottonseed meal	50			
	Nitrate of soda	354			
	Superphosphate	147			
	Muriate of potash	280			
	$MnSO_4$ (24% Mn)	85			
	Kieserite (29.65% MgO)	176			
6. Kieserite					
7. Sulfate of potash magnesia					

* Samples 1 and 2 and base for Sample 5 cured in 800 gram batches for 30 days at 60° C. with 7–10% moisture. Base for Sample 5 like that of Sample 2 but made from a less active Mg limestone, 15 B. Percentages of MgO corrected for loss of weight during curing and air-drying. The limestone in Samples 3 and 4 was finely ground, 72% passing a 200-mesh sieve.

3 had a considerable proportion of ammonium salts, which were omitted from Sample 4. Sample 5 was a cured base from the same series as Samples 1 and 2, but containing a less reactive magnesic limestone, and the 4–13.5–0 base was changed after curing to a 5–8–7 mixture by the addition of the ingredients listed, including kieserite and manganese. Samples 6 and 7 were kieserite and sulfate of potash magnesia, respectively, from commercial sources, but each had absorbed moisture during storage, causing lower percentages of magnesium oxide than is normal for these products.

Analyses were made of mixtures of the ingredients not expected to carry magnesium, and the quantities found were recalculated on the basis of the entire mixture. The corrections were greater than anticipated because of 1.18 per cent of magnesium oxide in the superphosphate that was used.

The procedures suggested are not published in detail because of the space required, but are described briefly as follows:

1. *Acid-soluble MgO.*—Proceeded as directed in *Methods of Analysis*, 1935, II, 54, and modified in 1936, *This Journal*, 20, 252 (1937).

2. *Water-soluble MgO.*—1 gram boiled for 60 minutes in 200 cc. of water. MgO was determined in a filtered aliquot by the Hoffman method used for acid-soluble MgO as noted above. It includes destruction of organic matter, removal of SiO_2, separation of $MgSO_4$ from $CaSO_4$ in alcohol, and reprecipitation of the $MgNH_4PO_4$ before ignition.

3. *Water-soluble MgO.*—1.25 grams boiled for 30 minutes in 200 cc. of water. The MgO was determined in a filtered aliquot by an abbreviated method suggested by Bartlett and Tobey (private communication). This method does not destroy organic matter or provide for dehydration of SiO_2. Ca is removed as the oxalate. Reprecipitation of the CaC_2O_4 and $MgNH_4PO_4$ is left to the discretion of the analyst, but is usually omitted. Periods of standing after precipitation are reduced to a minimum. This is a much more rapid procedure than the Hoffman method.

4. *MgO soluble in 2% NH_4Cl.*—1 gram boiled for 30 minutes in 200 cc of 2% NH_4Cl. The MgO was determined in a filtered aliquot by the Hoffman method.

5. *Citrate-insoluble MgO.*—1 gram treated as for citrate-insoluble P_2O_5, *Methods of Analysis*, 1935, II, 15, 16. This includes leaching with water and digestion with neutral ammonium citrate solution, sp. gr. 1.09. MgO is determined in the residue by the Hoffman procedure.

6. *MgO in water-soluble compounds, as kieserite and sulfate of potash magnesia.*—1 gram boiled for 30 minutes in 200 cc. of water. MgO was determined in a filtered aliquot by the Hoffman method or by the standard process of separation of Ca and Mg by ammonium oxalate.

Results were submitted by the following collaborators: Oscar I. Struve, Eastern States Cooperative Milling Corporation, Buffalo, N. Y.; H. D. Haskins and J. W. Kuzmeski, Agricultural Experiment Station, Amherst, Mass.; E. J. Deszyck, Agricultural Experiment Station, Kingston, R. I.; E. W. Magruder, F. S. Royster Guano, Norfolk, Va.; E. R. Tobey, Millard G. Moore, and C. Harry White, Agricultural Experiment Station, Orono, Me.

TABLE 2.—*Collaborators' results for MgO (per cent)*

COLLABORATORS	SAMPLES 1	2	3	4	5	KIESERITE	SULFATE OF POTASH MAGNESIA
	Method 1.—Acid-soluble MgO—Hoffman Method						
Struve	2.36	2.36	2.51	2.86	3.87		
Kuzmeski	2.58	2.45	2.55	2.92	3.81		
Deszyck	2.30	2.50	2.45	2.82	3.78	28.75	9.08
Magruder	2.44	2.39					
Moore	2.15	2.23	2.26	2.62	3.58		
Average	2.37	2.39	2.44	2.81	3.76		
Fertilizer base*	0.87	0.87	0.31	0.57	0.54		
Recovery of added MgO (%)	106	106	107	100	99		
	Method 2.—Water-soluble MgO—Hoffman Method						
Struve	1.81	1.01	1.10	1.87	2.87	28.92	8.69
Kuzmeski	1.97	1.16	1.38	1.88	3.03	28.84	8.87
Deszyck	1.83	0.98	1.19	1.80	2.97	28.79	9.00
Moore and White	1.93	1.11	1.03	1.73	2.85	28.87	8.88
Average	1.89	1.07	1.18	1.82	2.93	28.86	8.86
Fertilizer base*	0.69	0.69	0.23	0.41	0.44		
Recovery of added MgO (%)	85	27	48	63	76	100	98
	Method 3.—Water-soluble MgO—Bartlett-Tobey Method						
Struve	1.85	1.10	1.36	1.91	3.10		
Kuzmeski	1.82	1.04	1.30	1.86	3.01		
Deszyck	1.82	0.94	1.15	1.88	2.99		
Moore	1.86	0.97	1.06	1.77	3.39		
Average	1.84	1.01	1.22	1.86	3.12		
Fertilizer base*	0.70	0.70	0.24	0.41	0.45		
Recovery of added MgO (%)	81	22	49	64	82		
	Method 4.—MgO Soluble in 2% NH_4Cl—Hoffman Method						
Struve	1.88	1.07	1.56	2.07	3.00		
Kuzmeski	2.27	1.42	1.48	2.07	2.79		
Deszyck	1.77	1.04	1.39	2.02	2.96		
White	1.79	1.18	1.47	1.82	2.95		
Average	1.93	1.18	1.48	2.00	2.93		
Fertilizer base*	0.64	0.64	0.29	0.44	0.44		
Recovery of added MgO (%)	91	38	60	69	76		
	Method 5.—MgO insoluble in neutral NH_4 citrate						
Struve	0.72	1.63	1.09	1.05	1.05		
Kuzmeski	0.35	1.09	0.87	0.71	0.69		
Deszyck	0.32	1.09	0.74	0.76	0.67		

TABLE 2.—(*Continued*)

COLLABORATORS	SAMPLES 1	2	3	4	5	KIESERITE	SULFATE OF POTASH MAGNESIA
	MgO soluble in neutral NH_4 citrate, by difference						
Kuzmeski	2.23	1.36	1.68	2.21	3.12		
Deszyck	1.98	1.41	1.71	2.06	3.11		
Average	2.11	1.39	1.70	2.14	3.12		
Fertilizer base*	0.70	0.70	0.15	0.36	0.41		
Recovery of added MgO (%)	100	48	78	79	83		
	MgO insoluble in 2% citric acid, Method 5, but 2% citric acid in place of neutral NH_4 citrate						
Kuzmeski	0.24	0.24	0.11	0.18	0.35		
	MgO soluble in 2% NH_4Cl, Method 4, but Bartlett-Tobey procedure						
Kuzmeski	1.97	1.22	1.49	2.12	3.02		

* MgO found in the ingredients other than kieserite and Mg limestone by the different procedures, and calculated on the basis of the entire mixture.

Special credit should be accorded these collaborators for the excellent results reported and for their generous gift of time and attention to an unusually large assignment of analytical detail.

As may be noted (Table 2) the results for the acid-soluble and water-soluble fractions are very consistent. For the 2 per cent ammonium chloride extraction Kuzmeski obtained high results for three samples by the Hoffman method. He prefers the oxalate separation for use with this solvent, avoiding the precipitation of ammonium sulfate with calcium sulfate in the alcohol solution. By the Bartlett-Tobey oxalate separation he obtains results in better agreement with those reported by the other collaborators by the alcohol separation, indicating that the two methods may be made equally applicable for this solvent.

Two of the collaborators report concurring results for citrate-insoluble magnesium oxide, but those of the third analyst are consistently higher. The cause of the discrepancy has not been ascertained, but excellent agreement by the other analysts indicates that the procedure can be made to produce duplicate results satisfactorily.

Three of the samples contained manganese. This interfering element is not completely separated from magnesium by either the Hoffman or the Bartlett-Tobey methods, and the part that remains appears in the final precipitate as manganese pyrophosphate.

Hoffman[1] recommends that the manganese in the precipitate be determined by the colorimetric periodate method, and that the weight of the

[1] *Bur. Standards J. Research*, 9, 487–491 (1932).

precipitate be corrected in accordance with the results. This correction, reported by three collaborators, was in consistent agreement, and although it is a considerable portion of the precipitate, the correction seems sufficiently exact, as is shown by the satisfactory recovery of added magnesium oxide. No correction was required for manganese in the residue from the ammonium citrate or the citric acid extraction.

Despite certain theoretical objections that have been raised concerning the more rapid Bartlett-Tobey method, the results for water-soluble magnesium oxide (Table 2), and those reported last year for acid-soluble magnesium oxide, show satisfactory agreement with the longer Hoffman method. All the collaborators recommend the Bartlett-Tobey method, and the time that it would save as a routine method is of obvious importance. Unless authentic objections can be found under actual working conditions it should replace the Hoffman method as an official procedure.

SUPPLEMENTARY WORK

A study of the solubility of magnesium oxide in various common magnesic compounds used in fertilizers was continued from last year. Two mixtures described in the previous report, *This Journal*, 20, 252 (1937), were used. One was a 5–8–7 mixture made from common ingredients, but without ammoniation. The other was a 4–13.5–0 base con-

TABLE 3.—*Recovery of MgO from Mg limestone alone by NH_4 citrate solutions*

Mg limestone		Acid-soluble MgO	Recovery of MgO by NH_4 citrate	
			A, pH 4	B, pH 7
	mesh	*per cent*	*per cent*	*per cent*
12 A	80–100	18.88	97	20
3808	60–100	20.57	70	10
18	80–100	22.26	61	9
6	80–100	12.18	54	12
21	80–100	22.46	53	7
3	80–100	22.08	49	8
9	80–100	17.27	46	29
15 B	80–100	19.66	37	13

A. 0.2 gram 60 minutes 90°–95° C., in 100 cc. of 4% citric acid solution brought to pH 4 with NH_4OH.
B. 0.2 gram leached with water and treated with neutral NH_4 citrate solution, sp. gr. 1.09, in accordance with the official procedure for citrate-insoluble P_2O_5. In all cases, MgO was determined in the insoluble residue by the Hoffman method. The suspensions were shaken every 5 minutes.

taining superphosphate, sulfate of ammonia, and a dilute urea-ammonia liquor made in this laboratory. Magnesium carriers were added, and the mixtures were placed in a humid oven for 30 days at 60° C. The moisture was maintained at 5–10 per cent. The samples were air-dried after removal from the oven. These conditions probably cause a maximum reaction rate in relation to curing-pile conditions. The formulas and certain analyses presented previously are reported in Tables 4 and 5. Solubilities

of the magnesic limestones used, and others, are shown in Table 3. The data added this year show the relative effect of the combined water and neutral ammonium citrate extraction used exactly as in the determination of available P_2O_5, and the 4 per cent citric acid solution brought to *p*H 4 with ammonium hydroxide as described previously.

The results are expressed as the percentage recoveries of the magnesium

TABLE 4.—*Recovery of MgO from various Mg carriers added to a 5–8–7 fertilizer*[1] *by different solvents, and from the carrier alone*

MAGNESIUM CARRIER	RECOVERY OF MgO (PER CENT)				
	FROM 5–8–7 FERTILIZER NH_4 CITRATE			FROM CARRIER ALONE NH_4 CITRATE	
	A, *p*H 4	B, *p*H 7	C. WATER	D, *p*H 4	E, *p*H 7
Calcined kieserite	93	98	84		
Sulfate of potash magnesia	95	96	87		
$MgNH_4PO_4$	94	98	58		
Kieserite ½, sulfate of potash magnesia ½	98	97	89		
Kieserite ½, $MgNH_4PO_4$ ½	88	95	73		
$MgNH_4PO_4$ ½, sulfate of potash magnesia ½	92	96	71		
Magnesic limestones *mesh*					
12 A 100–120	83	0	4		
12 A 80–100	71	16	4	100	20
12 A 60– 80	77	0	3		
12 A 20– 60	59	0	4		
15 B 100–120	23	0	4		
15 B 80–100	19	7	4	60	13
15 B 60– 80	18	0	3		
15 B 20– 60	16	4	2		
9 80–100	31	3	5	55	29
21 80–100	24	3	2	52	7
3 80–100	20	0	3	59	8
12 A 80–100 ½, kieserite ½	85	55	41		
12 A 80–100 ½, sulfate of potash magnesia ½	86	61	45		
12 A 80–100 ½, $MgNH_4PO_4$ ½	73	51	36		
Serpentine	75	30	22		
Olivine	30	29	12		

[1]

Base mixture:	*lbs.*	*Base mixture:*	*lbs.*
Sulfate of ammonia	250	Diammonium phosphate	120
Nitrate of soda	100	Superphosphate	600
Cottonseed meal	126	Muriate of potash	280

Magnesium carriers supplied 3% MgO in the mixture; 1500 grams of each mixture was cured for 30 days at 60° C. and with 5–7% moisture. The calculations take into account the MgO in the base mixture and weight changes during air-drying.

A = 1 gram with 100 cc. of 4% citric acid adjusted to *p*H 4 with NH_4OH, 90 min., 90–95° C, with shaking every 5 minutes.
B = As for citrate-insoluble P_2O_5, official method, 60 minutes.
C = Boiled 1 gram with 150 cc. of water for 30 minutes.
D = Like A, with 0.2 g. sample.
E = Like B, with 0.2 g. sample.

oxide added in the different carriers. In each instance the magnesium oxide present in other ingredients, such as the phosphates, potash, and nitrogen salts, was determined in a separate mixture from which the other magnesic compounds were omitted. These mixtures were treated and analyzed in the same way as were the others, and the results obtained were subtracted after calculation on the basis of the entire mixture. Changes in the weight of each mixture from carbon dioxide and moisture losses were also taken into account in the calculations.

TABLE 5.—*Recovery of MgO from various carriers added to a 4–13.5–0 base*[1] *by different solvents*

MAGNESIUM CARRIER		RECOVERY OF MgO (PER CENT)		
		NH_4 CITRATE[2]		WATER
		pH 4	pH 7	
Calcined kieserite		98	95	85
Magnesic limestones	*mesh*			
12 A	100–120	84	58	38
12 A	80–100	86	42	26
12 A	60– 80	81	44	25
12 A	20– 60	87	43	34
15 B	100–120	60	28	31
15 B	80–100	35	18	16
15 B	60– 80	54	29	30
15 B	20– 60	32	7	13
3	80–100	43	21	22
21	80–100	47	25	19
22	80–100	40	13	—
Olivine		52	24	35

[1]

Base mixture:	*lbs.*
Superhphosphate	1500
Urea-ammonia liquor (25.7% N)	242
Ammonium sulfate	98

Magnesium carriers supplied 1.3% MgO in the mixture, 800 grams of each mixture was cured for 30 days at 60° C., 7–10% moisture. Calculations take account of MgO in the base, and weight changes from decomposition and moisture loss on air-drying.

[2] Solvents and details described in footnote of Table 4, also the MgO recoveries from the limestone alone.

A third group of mixtures was prepared in order to study the individual effect of common non-magnesic fertilizer ingredients on the solubility of magnesium oxide in common carriers. A 5–8–7 fertilizer was formulated from the 7 ingredients noted in Table 4. Mixtures were made in accordance with the proportionate parts of the formula, but from each mixture one ingredient was omitted. A single carrier of magnesium oxide was added to portions of each of these mixtures to make 3 per cent of magnesium oxide on the basis of the complete original formula. Magnesium

ammonium phosphate was included, not as a common carrier of magnesium oxide, but because it is probably a frequent reaction product in cured fertilizer mixtures. The serpentine and olivine were taken from demonstration shelves, and may not be strictly representative of large deposits.

The samples were ground to pass a 1 mm. sieve and analyzed immediately by methods already indicated in the previous experiments. Each ingredient was analyzed separately for each solvent, and corrections for magnesium oxide in the superphosphate, cottonseed meal, etc. were made in calculating recoveries of magnesium oxide from the principal magnesium compounds.

The data in Table 6, therefore, represent the effect of solvents on dry mixtures, and the reactions noted must be ascribed very largely to changes caused by the analytical technic.

Relationships among the three solvents are discussed under another section, but it may be noted in Table 6 that only the superphosphate and sulfate of ammonia appear to have a significant effect upon the recoveries. Taking analytical errors into account, the differences are small, but there is a trend toward greater recovery of magnesium oxide from magnesium limestones and magnesium ammonium phosphate in the acid ammonium citrate solution where superphosphate was not present, and lower recoveries when sulfate of ammonia is omitted. Reactions with superphosphate increased the apparent solubility of magnesium ammonium phosphate in water. None of the ingredients made significant changes in the recovery of magnesium oxide from kieserite.

Since these analyses were completed, the samples have been oven-treated at 60° C. for 30 days with 7 per cent moisture. The analyses now underway are expected to show greater differences as a result of reaction among the ingredients.

DISCUSSION OF RESULTS

As has been emphasized in previous reports, a method for "active" or "available" magnesium oxide must have the backing of correlation with plant response. S. F. Thornton of the Indiana Experiment Station has undertaken a research project intended to supply this information, but the results are not yet ready for publication. In the meantime, various solvents are being studied as an aid in selection of the most promising for intensive work when the relative values of the various compounds are known more definitely.

The compounds expected to be important, either as added ingredients or as reaction products, may be grouped according to the degrees of activity anticipated as follows: Very available—magnesium sulfate, double sulfate of potash magnesia, the phosphates of magnesia, and magnesium ammonium phosphate; those probably less available, but degree of

TABLE 6.—*Effect of individual fertilizer ingredients on the recovery of MgO from Mg carriers in a 5–8–7 fertilizer*[1]

CARRIERS	RECOVERY OF MgO (PER CENT)[3]									
	NH_4 CITRATE, pH 4				NH_4 CITRATE, pH 7				WATER	
	Mg LIMESTONES				Mg LIMESTONES					
	80–100 MESH		$MgNH_4PO_4$	KIESERITE	80–100 MESH		$MgNH_4PO_4$	KIESERITE	$MgNH_4PO_4$	KIESERITE
	12A	15B			12A	15B				
Ingredient omitted[2]										
$NaNO_3$	82	30	98	99	16	11	99	98	73	98
KCl	86	35	94	95	26	16	99	98	82	94
Cottonseed meal	83	31	100	100	25	13	99	98	77	94
$(NH_4)_2SO_4$	72	25	92	99	25	10	98	99	78	95
$(NH_4)_2HPO_4$	82	32	97	100	27	15	99	98	84	95
Superphosphate	94	37	103	100	19	15	99	99	63	95
Sand	85	31	97	99	23	8	99	99	81	96
Superphosphate and $(NH_4)_2HPO_4$	92	44	100	101	26	11	99	98	75	100

[1] Ingredients described in footnote of Table 4.
Mg carriers supplied 3% MgO. Sand added to make 2000 lbs. Ingredients mixed in normal moisture state and not oven cured.
[2] From each of 7 batches one ingredient was omitted, and from the 8th lot all phosphates were left out. Recoveries are calculated on the uniform basis of 2000 lbs. and are corrected for MgO from ingredients not intended to supply Mg.
[3] Details of extractions are described in Table 4.

activity uncertain—dolomitic limestones, and natural silicates as olivine, serpentine, and talc.

Acid-Soluble Magnesia.—The hydrochloric-nitric acid mixture used in many laboratories for the dissolving of total phosphoric acid seems equally satisfactory for magnesia. Possibly fusion methods may cause slightly higher results by more complete decomposition of silicates, but it is unlikely that magnesia insoluble in these strong acids is useful to plants within a reasonable time. The method has shown satisfactory agreement among different analysts for three years of collaboration, and the recovery of magnesia from mixtures of known formulas has been good (Table 2), even when manganese salts are present. Unfortunately the procedure is too long to be well adapted to routine work.

Water-Soluble Magnesia.—This fraction is usually conceded to be highly available, whether it results from water-soluble ingredients, or water-soluble products from reactions while curing or during storage. Certain states, notably Maine,[1] accept guarantees for this fraction, as well as for total magnesia, and manufacturers appear to meet these guarantees as consistently as any others. The objection is that several compounds that are not water-soluble are expected to prove readily available, and these compounds should not be excluded by a method interpreted as measuring the active fraction of magnesia.

Data in this and previous reports indicate that magnesia from kieserite and sulfate of potash magnesia is recovered almost completely from dry, uncured fertilizers by boiling with water, but that the recovery is somewhat less from cured mixtures. The apparent decrease in solubility is not great, for the recoveries exceed 85 per cent. If the water-soluble magnesia in all the ingredients, superphosphate, potash salts, etc., is included, as would be the case under practical conditions, there probably would be no apparent loss from added water-soluble compounds.

The formation of water-soluble compounds of magnesium from reactions of other fertilizer ingredients with dolomite and magnesic limestones has been slight where ammonia liquors were not used, but reactions with these liquors were more effective. Data in Table 5 show recoveries varying from 13 to 38 per cent of the small quantity of magnesia added in limestones. The maximum would represent 0.5 per cent of water-soluble magnesia on the basis of this mixture.

In an important contribution to this topic, Beeson and Ross[2] show similar data for a greater range of temperature and time of storage for a single dolomite used at three different rates and in four mixtures. The data indicate that the principal reaction products are di-magnesium phosphate with non-ammoniated mixtures and magnesium ammonium

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[1] Official Inspections 161, Maine Agricultural Experiment Station (1936).
[2] *Ind. Eng. Chem.*, 29, 1176 (1937).

phosphate where ammonia liquors are used, and that neither type of reaction yields important quantities of water-soluble magnesia.

Data in the previous report show greater recovery of magnesia by boiling with water than by leaching. An important criticism has been suggested by H. C. Moore,[1] who points out that alkaline extracts encourage the formation of magnesium ammonium phosphate, and thus reduce the recovery of magnesia originally water-soluble. Immediate filtration and acidification of the extract prevent this. W. H. MacIntire suggests a somewhat similar criticism of changes brought about by analytical manipulation, rather than by storage reactions, and has provided the writer with samples of four mixtures to illustrate his suggestion. Two of the

TABLE 7.—*Solubility of magnesia in mixtures of monocalcium phosphate, dolomite, and selectively calcined dolomite*[1]

SAMPLE	ACID-SOLUBLE MgO	MgO SOLUBLE IN NEUTRAL NH_4 CITRATE	WATER-SOLUBLE MgO
	per cent	*per cent*	*per cent*
Mixture 1—			
1000 lbs. dolomite	12.59	3.19	0.92
500 lbs. treble-superphosphate			
Wet mixed			
Mixture 2—			
Like Sample 1, but dry mixed	12.52	2.88	0.95
Mixture 3—			
200 lbs. selectively calcined dolomite 355.6 lbs. $CaH_4(PO_4)_2$	9.44	9.26	2.13
Wet mixed			
Mixture 4—			
Like Sample 3, but dry mixed	10.67	10.55	2.46

[1] Received from W. H. MacIntire, Tenn. Agr. Exp. Station. Samples 1 and 2 were mixed in May, and Samples 3 and 4 in October, 1935. Extractions were made as described in Table 4, water-soluble fraction by boiling 1 gram 60 minutes in 200 cc.

mixtures contain dolomite and treble superphosphate, one mixed dry and the other in water suspension. Two other samples have monocalcium phosphate, and dolomite heated to decompose the magnesium carbonate to magnesium oxide, leaving the calcium carbonate largely undecomposed. This material, called selectively calcined dolomite, is insoluble in water, as is the untreated dolomite. Analyses in the writer's laboratory (Table 7) show little formation of water-soluble magnesium compounds from the dolomite in proportion to the quantities of magnesia present, but much greater reactivity for the calcined dolomite. If the reactions proceed during a storage period, the effect of the wet mixing should be evident in greater changes than in the dry samples. The fact that such differences do not appear may indicate that the products dissolved may have formed during the boiling in the laboratory.

[1] Private communication.

The importance of these criticisms depends, in the opinion of the writer, upon the objectives of the work. If the object is to identify and measure an ingredient that has been added to a mixture, it is very important; but if the desire is to create a laboratory procedure to correlate with plant response, the increased activity of more reactive products may prove beneficial. The matter is important and will receive further study.

TABLE 8.—*Summary of the recoveries of MgO by neutral ammonium citrate from fertilizers*
(Results expressed as per cent of the MgO added in Mg limestones)

FERTILIZER MIXTURE	Mg LIMESTONE INCORPORATED IN FERTILIZER		RECOVERY
	Fineness	*MgO per Ton lbs.*	*per cent*
Sample 3, Table 2, uncured, has NH_4 salts	12 A, mill run, fine	20	56[a]
Sample 4, Table 2, uncured, no NH_4 salts	12 A, mill run, fine	15	39[a]
	mesh		
5–8–7, Table 6, uncured, not ammoniated	12 A, 80–100	60	23[b]
5–8–7, Table 6, uncured, not ammoniated	15 B, 80–100	60	12[b]
5–8–7, Table 4, cured 60°C., 30 days, not ammoniated	12 A, 80–100	60	16
5–8–7, Table 4, cured 60°-C., 30 days, not ammoniated	15 B, 80–100	60	7
3–9–5, Beeson and Ross,[c] cured 60°C., 90 days, not ammoniated	Finer than 30	32	42
6–18–10, Beeson and Ross,[c] cured 60°C., 90 days, not ammoniated	Finer than 30	32	40
3–9–5, Beeson and Ross,[c] cured 60°C., 90 days, ammoniated	Finer than 30	32	62
6–18–10, Beeson and Ross,[c] cured 60°C., 90 days, ammoniated	Finer than 30	32	74
4–13.5–0, Table 5, cured 60°C., 30 days, ammoniated	12 A, 80–100	26	42
4–13.5–0, Table 5, cured 60°C., 30 days, ammoniated	15 B, 80–100	26	18
Sample 5, Table 2, cured 60°C., 30 days, ammoniated	15 B, 80–100	12	14[a]

[a] MgO added in kieserite and non-magnesic ingredients subtracted before recovery was calculated.
[b] Average of several mixtures.
[c] *Ind. Eng. Chem.*, **29**, 1176 (1937). Results calculated from published curves for mixtures containing 190 lbs. of limestone and furnishing 1.62 per cent of MgO to the mixture.

Citrate-Soluble Magnesia.—The use of citrate solutions for active soil elements and available phosphoric acid in fertilizers suggests its possible adaptability to this problem. Last year the solubility of magnesia from common carriers in two series of mixtures was determined by extraction with 4 per cent citric acid adjusted to pH 4 with ammonium hydroxide. No theoretical background is claimed for this solution, but it seemed to be the proper type and could serve as a standard of comparison for other modifications of acidity, concentration, time of extraction, etc. It is

about 4.5 times as concentrated with respect to the citrate radical as the official citrate solution for phosphates.

During the year several analysts suggested the use of the combination of leaching with water and extraction with neutral ammonium citrate now used for available phosphoric acid (P_2O_5). W. H. MacIntire suggested that it should dissolve all water-soluble compounds of magnesium, and also the water-insoluble phosphates. Thus, it would include the more reactive portions of dolomite in base goods, particularly ammoniated mixtures, for these are changed to phosphates. Trials of the procedure have verified this expectation. The difficulty lies not with the compounds mentioned, but with the evaluation of dolomite residues in base goods and in dry mixtures that have had little opportunity to react.

Certain pertinent comparisons are shown in Table 8. This summary should be considered only in addition to all the other data in this report and in the publication by Beeson and Ross, cited above, for the selection is restricted to what seem to be the most important cases, and may be inadequate. It is intended to show the general magnitude of recoveries that have been found for the various conditions.

The following observations indicate present knowledge of the application of the neutral citrate solution to the problem:

1. Magnesic limestones vary in reactivity.
2. Particle size and ratio of weight of charge to volume of solvent are factors in reactivity.
3. Water-soluble carriers of magnesia, and magnesium ammonium phosphate are recovered by this solvent.
4. Recovery of magnesia from dry mixtures varied from 12 to 56 per cent, indicating dependence on variable conditions.
5. Oven-curing of an unammoniated 5–8–7 mixture did not increase the recovery of magnesia.
6. Ammoniation of base goods increases magnesia recovery, but under different conditions the portions recovered varied from 14 to 74 per cent.
7. In a dry mixture the solubility of two limestones was approximately equal to the solubility of the limestones alone if the ratios of weights of limestone to volume of solvent were similar, but this agreement was not found after the same unammoniated mixtures were cured. Ross and Beeson found increased solubility for their mixtures, while small decreases were found for a mixture studied in this laboratory.
8. Although very variable, most of the recoveries from unammoniated mixtures seem lower than the decomposition to be expected from small quantities of magnesic limestone in average soils, hence probably too small to represent availability.

In view of the uncertainty it seems best to postpone decision regarding the usefulness of this solvent until the results of pot tests can be compared.

Magnesia Soluble in 2 per cent Ammonium Chloride, and 2 per cent Citric Acid.—The ammonium chloride solvent, suggested by H. D. Has-

kins, is intermediate between water and neutral ammonium citrate solution. It probably dissolves all water-soluble carriers and makes less attack on limestone residues than the neutral ammonium citrate. Two per cent citric acid makes a strong attack on all of the mixtures where it has been used.

As has been stated for the neutral ammonium citrate, the usefulness of these methods also must await the determination of crop response in pot cultures.

Magnesia in Water-Soluble Compounds.—It was shown last year that the magnesia in the double sulfate of potash magnesia may be extracted completely by boiling in water, but not by leaching on a filter with the customary volumes of water. A procedure suggested then for the determination of magnesia in this product, kieserite, and sulfate of magnesia was tested in four laboratories this year, and satisfactory results were obtained. Originally the method was written to be used with either the oxalate or the alcohol modifications, but the former is shorter and the alcohol separation of calcium sulfate is omitted in the method recommended. This method was published in *This Journal,* **21,** 77 (1938).

MANGANESE IN FERTILIZERS

Several methods were tried with pyrolusite, commercial manganese sulfate, and four fertilizer mixtures. The results (Table 4) indicate fair agreement with compounds of manganese, but difficulties were found

TABLE 9.—*Results on manganese by a volumetric periodate and other methods*
(Results expressed as per cent Mn)

MATERIAL	VOLUMETRIC-PERIODATE METHOD			BISMUTHATE METHOD	FORD-WILLIAMS METHOD	VOLHARD METHOD
	ANALYST					
	1	2	3			
Pyrolusite	53.75	—	—	54.30	52.98	54.01
Commercial manganese sulfate	24.15	24.18	—	24.49	23.40	24.94
7-6-6, Sample 3[a]	1.00	1.06	—	—	—	—
5-9-8, Sample 4[a]	1.02	0.98	—	—	—	—
5-8-7, Sample 5[a]	1.01	1.02	—	—	—	—
Sample 6[b]	2.39	2.33	2.27[b]	—	—	—

[a] Samples are described in Table 1 and are calculated to contain 1% Mn
[b] Mixture, containing superphosphate, ground tobacco stems, commercial manganese sulfate, and sand, and analysis furnished by F. B. Carpenter, Virginia-Carolina Chemical Corporation.

with certain of these procedures when used with the fertilizer mixtures. The bismuthate method[1] failed to give a stable end point. The Ford-Williams[1] method gave low results for manganese compounds. This

[1] Standard Methods of Chemical Analysis. Wilfred W. Scott, 4th ed. revised, p. 302, 303, 306 (1925).

method utilizes potassium chlorate in nitric acid as an oxidizing agent and requires very careful manipulation. The Volhard method did not give a distinct end point, and is longer than the volumetric periodate method suggested to the Associate Referee by F. W. Carpenter.[1]

The periodate method is a volumetric adaptation of the well-known Willard and Greathouse[2] colorimetric procedure for manganese. It is a relatively simple method and gave good agreement in the hands of two analysts in this laboratory, and with the analysis furnished for one mixture by F. W. Carpenter. The only difficulty noted was slow filtration of the mercuric precipitate of the excess salts of iodine.

The procedure will be studied further before the presentation of a tentative method, but the details are published here in the hope of stimulating trials and comparisons in other laboratories.

ACID-SOLUBLE MANGANESE IN FERTILIZERS AND MANGANESE SALTS

Weigh 1 gram of sample into a beaker, and add 30 cc. of HNO_3 and 10 cc. of HCl. Cover the beaker with a cover-glass supported above the rim of the beaker and digest at the boiling point for 30 minutes, adding more of the acids to prevent evaporation to dryness. To the solution, or to an aliquot if more than 30 mg. of Mn is present, add 15 cc. of a mixture of acids consisting of one part of H_2SO_4, 7 parts of 85% H_3PO_4, and 7 parts of water, by volume, and evaporate to white fumes in the covered beaker. If organic residues remain at this point, add successive portions of 5 cc. of HNO_3, repeating the evaporation after each addition until the organic matter is destroyed, and chlorides are dispelled. Dilute to a volume of 50–75 cc. with water. Add KIO_4 at the ratio of 0.3 gram for each 15 mg. of Mn, either as the dry salt, or more conveniently from a solution of known concentration. Avoid a large excess to save time in filtration at a later stage. Heat at the boiling point for 30 minutes. Dilute to 150 cc. and cool. Add 25 cc. of 10% $Hg(NO_3)_2$, made by dissolving 10 grams of $Hg(NO_3)_2$ in 90 cc. of water, and clearing with a few drops of HNO_3. Stir four times within 3 minutes and filter the precipitate of iodine salts on a thick pad of asbestos on a Gooch crucible, using suction. Wash with water until the washings are no longer pink. Reduce the $KMnO_4$ in the filtrate with an accurately measured volume of 0.0910 *N* $FeSO_4$, using a small excess. Titrate the excess $FeSO_4$ with 0.0910 *N* $KMnO_4$. 1 cc. of 0.0910 *N* $FeSO_4$ = 1 mg. of Mn.

STANDARD SOLUTIONS

Potassium permanganate.—0.0910 *N*. 2.876 grams of $KMnO_4$ in 1 liter of solution. Standardize with Na oxalate.

Ferrous sulfate.—0.0910 *N*. 25.3 grams of $FeSO_4 \cdot 7H_2O$ in 1 liter of solution. Standardize with the 0.0910 *N* $KMnO_4$.

RECOMMENDATIONS[3]

It is recommended—

(1) That the method entitled "Total Magnesia," *Methods of Analysis, A.O.A.C.*, 1935, II, 54, be changed to read "Acid-Soluble Magnesia,"

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[1] Private communication.
[2] *J. Am. Chem. Soc.*, 39, 2366 (1917).
[3] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 61 (1937).

modified in minor details last year, and adopted (first action), *This Journal*, **20**, 51, 67 (1937), be adopted as official (final action).

(2) That the method recommended in this report for the determination of magnesia in water-soluble compounds used as sources of magnesium in mixed fertilizers, be adopted as a tentative method. See *This Journal*, **21**, 77 (1938).

(3) That the study of methods for active magnesia in mixed fertilizers be continued.

(4) That the method for acid-soluble manganese presented in this report be studied collaboratively, and that other methods for manganese be compared.

REPORT ON POTASH

By O. W. Ford (Purdue University Agricultural Experiment Station, West Lafayette, Ind.), *Associate Referee*

No collaborative work was done on potash referee work in 1937, but the following questionnaire was sent out to 37 experiment stations and commercial chemists:

In connection with A.O.A.C. referee work on potash for 1937 it was recommended that the following points be considered.

1. The matter of providing for use of a factor weight.

Do you think it would be advantageous to provide for a factor weight?

2. The elimination of the barium chloride method for the determination of potash in fertilizers.

Do you see any valid reason why this method should not be eliminated?

3. A method of recovery of platinum used in the determination of potash.

Will you please send me a copy of the method of recovery that you use in your laboratory?

COMMENTS ON THE QUESTIONNAIRE

Of the 23 replies received, 15 represented the views of Experiment Station chemists; 8 of these favored and 7 did not favor the use of factor weights. Eight replies were received from commercial chemists. These were divided 6 for and 2 against the use of factor weights. Thus a summary of the replies would indicate that 14 favored and 9 did not favor the use of factor weights. From the interest shown it is evident that some collaborative work should be done in determining a factor or a set of factor weights.

As no reply received favored the retention of the barium chloride method and as many strongly suggested that it be dropped it will be so recommended.

A summary of the comments on the method of recovery of platinum revealed that, of the 23 chemists reporting, almost without exception there was an expression in favor of the adoption of some standard pro-

Comparison of the official and a modification of the official method for potash designed to prevent foaming in preparation of the solution

SAMPLE NO.	K_2O GUARANTEE	OFFICIAL METHOD	MODIFIED* OFFICIAL METHOD	DIFFERENCE + OR − FROM OFFICIAL	FILTRATION BEFORE PRECIPITATION WITH PLATINUM SOLUTION		DIFFERENCE + OR − FROM	
					OFFICIAL	MODIFIED* OFFICIAL	OFFICIAL	MODIFIED* OFFICIAL
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2	2.66	2.56	−.10	2.21	2.13	−.45	−.43
2	4	5.12	5.36	+.24	4.98	4.93	−.14	−.43
3	6	6.88	6.80	−.08	6.71	6.58	−.17	−.22
4	4	5.06	5.11	+.05	4.58	4.58	−.48	−.53
5	8	8.47	8.59	+.12	8.14	8.27	−.33	−.32
6	10	11.11	11.08	−.03	11.12	11.20	+.01	+.12
7	8	8.56	8.52	−.04	8.72	8.78	+.16	+.26
8	6	6.86	6.78	−.12	6.57	6.55	−.19	−.23
9	14	12.88	13.14	+.26	12.92	13.12	+.04	−.02
10	10	10.92	10.86	−.06	10.64	10.52	−.28	−.34
11	12	12.14	12.24	+.10	11.94	11.82	−.20	−.30
12	3	3.47	3.60	+.13	3.16	3.10	−.31	−.50
13	12	12.24	12.22	−.02	12.24	12.23	±.00	+.01
14	24	15.64	16.14	+.50	15.36	16.13	−.28	−.01
15	12	11.16	10.96	−.20	11.38	11.12	−.22	+.16
16	20	20.04	19.78	−.26	20.16	19.90	+.12	+.12
17	20	20.38	20.16	−.22	20.38	20.06	±.00	−.10
18	16	15.28	15.70	+.42	15.24	15.69	−.04	−.01
19	32	25.44	24.95	−.49	25.46	24.82	+.02	−.13
20	18	17.30	17.06	−.24	17.40	17.06	+.10	±.00
Maximum	32	25.44	24.95	+.50	25.46	24.82	−.48	−.53
Minimum	2	2.66	2.56	−.49	2.21	2.13	+.16	+.26
Average	12.05	11.58	11.58	±.00	11.47	11.43	−.13	−.15

* Modified official method—5 gram sample, 500 ml. flask, 200 ml. water, 80 ml. saturated ammonium oxalate.

cedure or procedures. Closer scrutiny of the reports showed that 16 of the 23 were at present using some form of zinc, 3 were using aluminum, and 4 were using a combination of one or two various other methods to accomplish the reduction of the potassium chloroplatinate salt to platinum black. Of those reporting 8 stated that they were fairly well satisfied with the method of recovery that they were using; 11 stated that they sometimes were in doubt as to the purity of the recovered platinum, while 4 stated that the method they were using was not satisfactory and that they would like to see a simple procedure worked out and proposed for adoption. From these expressions it is evident that there is a need for such a method, and the Associate Referee on Potash will recommend that further study be made in order that a method or methods of recovery can be worked out and proposed for adoption.

The data in the table deal with two recommendations suggested for consideration in the 1937 referee work on potash; the first relates to "filtration of the potash solution after ignition and solution if necessary," and the second to "the problem of foaming during the boiling of the sample in the present official method in the determination of potash."

Both sets of data in this table were obtained from different aliquots of the same sample solution. Out of more than 100 samples that were tested in the laboratory for foaming, this set of 20 samples, ranging in potassium oxide content from 2 to 32 per cent, was chosen. Sample 18 had to be weighed out six times and watched very closely before a solution was obtained that did not foam over while being heated. All solutions were put on a cold sand bath and brought to the boiling point for the required period. Although most of these showed a marked tendency to froth over when the present official method was used, no trouble with foaming was encountered with the modification of the official method that was tried out. The results of the modification of the official method, with the increase to a 5 gram sample, should offset to a small extent the feeling that a non-uniformity of sample exists when only a 2.5 gram sample is weighed out.

Many chemists have occasionally found insoluble residues after ignition and solution even when platinum dishes were used for ignition. The potassium oxide results reported were run from the same sample solutions by filtering, after ignition and solution, before precipitation with the platinum solution. The results of the data from this standpoint warrant further study, and should this procedure later prove to be sound there would be no necessity for further investigation of Section **44**, (a), p. 30, "Weigh and remove the chloroplatinate precipitate by washing with hot H_2O, using slight suction. Wash with 80% alcohol three times, dry as before, and weigh (loss equals K_2PtCl_6). Calculate to K_2O."

Attention of the Associate Referee was called to the fact that too long a contact of the acid alcohol with the potassium chloroplatinate may result in low results. Further that the solvent action was increased with the increase of the volume of alcohol and by length of time left in contact with the acid alcohol.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of the use of a factor weight or of factor weights in the determination of potash in fertilizers be continued.

(2) That the barium chloride method for the determination of potash, given in paragraphs **45, 46,** and **47,** page 31, *Methods of Analysis, A.O.A.C.* 1935, be deleted (first action).

(3) That further study be made of the determination of potash by

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 61 (1938).

filtration after ignition and solution when platinum or silica dishes are used.

(4) That further study be made of the need for providing additional platinum solution concentrations.

(5) That a study of the methods of the recovery of platinum be made with a view to recommending the adoption of one or more procedures.

(6) That additional investigation be made of some modification of the present official method to prevent foaming during the boiling of the sample.

(7) That additional studies be made of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination (p. 30, **43**).

(8) That the solvent action of acid alcohol on potassium chloroplatinate be studied.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to H. R. Kraybill for his counsel and to C. W. Hughes for assistance in making the potash determinations herein enclosed.

REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS

By L. E. Horat (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

During the past year further studies were conducted in accordance with the recommendations of the Association, *This Journal*, **20**, 51 (1937), with special reference to the tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 34–35, and modifications submitted by the Associate Referee, *This Journal*, **20**, 264–275 (1937).

In view of the large number of collaborators last year and the excellent data submitted by them, *Ibid.*, only six fertilizer samples were sent to W. H. Pierre and C. L. Hare for collaboration. It was deemed advisable also to offer more detailed experimental evidence in support of several points brought out by last year's collaborative results and briefly referred to, such as the proper end point of titration and proper indicator for titration to that end point.

END POINT OF TITRATION

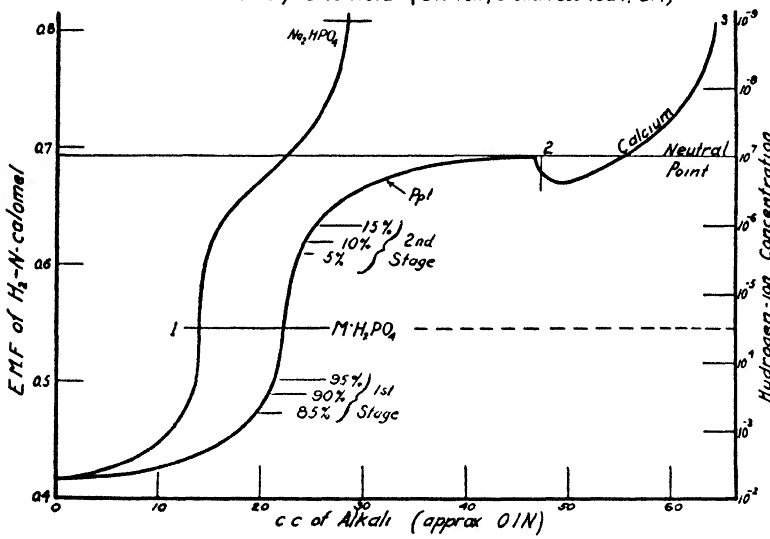
In the determination of acid- and base-forming quality of fertilizers by the tentative method the difficulties encountered in the titration (with indicator) of the filtrate of acidified ash have been referred to before by

Smith, *This Journal*, **18**, 221–37 (1935), and by Horat, *Ibid.*, **20**, 264–275 (1937).

The work of Britton[1] on an electrometric study of the precipitation of phosphates was mentioned in last year's report as a possible explanation of difficulties that may be encountered when ash solutions of phosphate fertilizers are titrated to an end point above *p*H 4.5.

Fig. 1 was reproduced from Britton's paper to show the similarity in type of the curves obtained by electrometric titration of phosphoric acid with sodium hydroxide and calcium hydroxide and the curves obtained by electrometric titration of the fertilizer samples in Figs. 2 and 3.

Fig 1 - Hydrogen Electrode Titration Curves of Phosphoric Acid (Britton, J Chem Soc 1927, 614)



In Fig. 1, 100 cc. of 0.01277 *M* orthophosphoric acid was titrated with 0.01919 *N* sodium hydroxide and 100 cc. of 0.01024 *M* orthophosphoric acid was titrated with 0.0447 *N* calcium hydroxide. Britton stated that the points above the titration of the first hydrogen of phosphoric acid with calcium hydroxide represented immediate and not equilibrium values. In Figs. 2 and 3 all points plotted more nearly represent equilibrium values than immediate values, inasmuch as one to two minutes elapsed after each addition of alkali before *p*H readings were taken.

The fertilizer samples whose titration curves are plotted in Figs. 2 and 3 had the composition shown in Table 1.

Precipitated phosphate, the by-product of baking powder manufacture, was used as the source of phosphate in all samples in Table 1 except

[1] *J. Chem. Soc.*, Papers, Pt. I, 614 (1927).

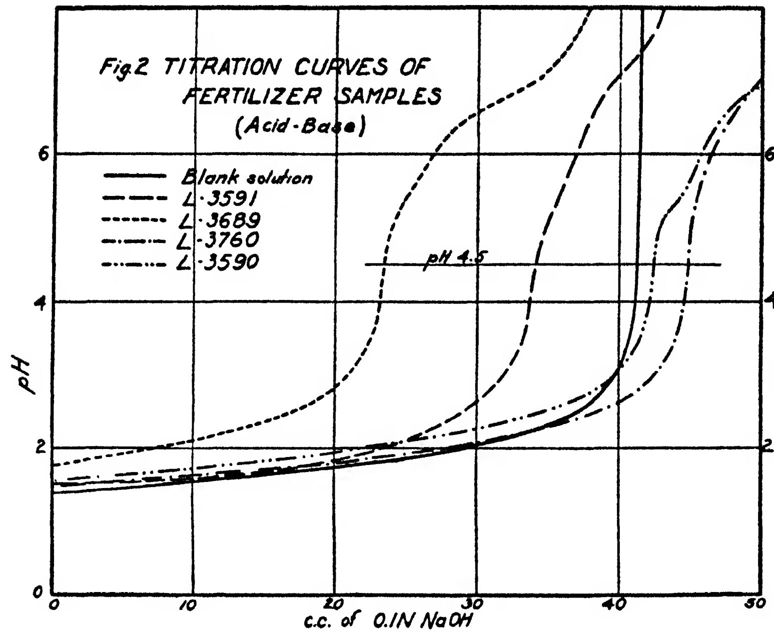


Fig. 2 TITRATION CURVES OF
FERTILIZER SAMPLES
(Acid-Base)
Blank solution
L-3591
L-3689
L-3760
L-3590
pH 4.5
pH
0
2
4
6
0
10
20
30
40
50
c.c. of 0.1N NaOH

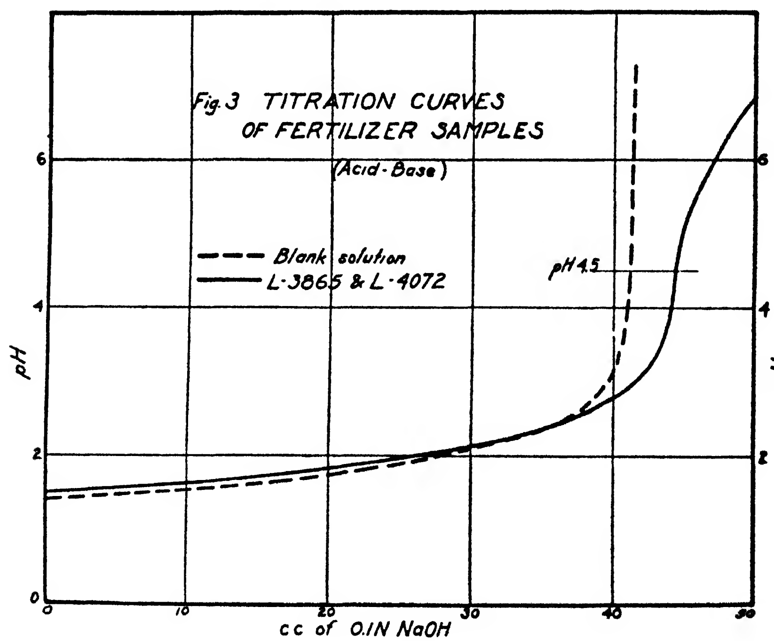


Fig. 3 TITRATION CURVES
OF FERTILIZER SAMPLES
(Acid-Base)
Blank solution
L-3865 & L-4072
pH 4.5
pH
0
2
4
6
0
10
20
30
40
50
cc of 0.1N NaOH

TABLE 1.—*Fertilizer samples*

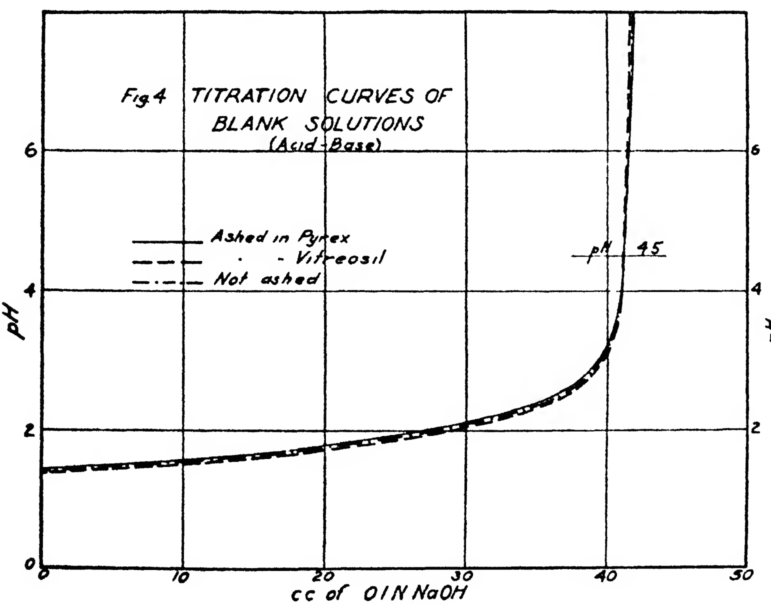
SAMPLE NO.	ANALYSIS	TOTAL N FOUND	INSOLUBLE P_2O_5 FOUND	TOTAL ACIDITY CORRECTION
		per cent	*per cent*	*lbs.* $CaCO_3$
L-3590	2-12- 6	2.2	1.2	112
L-3591	2- 8-16	2.0	0.6	88
L-3689	2-12- 2	2.1	1.7	123
L-3760	4-24-12	4.2	1.0	178
L-3865	4-24- 4	4.0	1.0	171
L-4072	4-24- 4	4.0	1.5	185

L-3760, in which superphosphate was used. The presence of a considerable amount of aluminum and iron phosphates in the precipitated phosphate has an effect on the precipitation of calcium phosphate in the titration as described by Britton, who found that the presence of aluminum salts lowered the *p*H at which precipitation of the calcium of the calcium phosphate began.

By consideration of the concentrations of acid and alkali used by Britton and the comparatively larger concentrations used in titration of the ashed fertilizer solutions the increased buffering effect above *p*H 4.5 indicated in Figs. 2 and 3 can be better understood.

SOLVENT ACTION OF SODIUM CARBONATE AT 600° C.

In regard to the tentative method for determining the acid- and base-forming quality of fertilizers the question has arisen as to possible solvent



action of sodium carbonate solution on various types of vessels used for ashing the sample at 600° C. for one hour.

An effort to determine this solvent action was made in this laboratory by ashing, as prescribed in the tentative method, with blank solutions of 10.0 cc. of molar sodium carbonate solution containing sucrose in Pyrex and in Vitreosil beakers. The ashed residues were acidified, carbon dioxide was removed according to the tentative method, and solutions were made up to 250 cc. volumes. Aliquot portions of 100 cc. were then titrated with 0.1 *N* sodium hydroxide electrometrically with a Beckman *p*H meter. Un-ashed blank solutions were titrated in a similar manner.

The results obtained are illustrated graphically in Fig. 4. By interpolation the values of ashed and un-ashed blank solutions at *p*H 4.5 were calculated.

	cc.	
Blank solution ashed in Pyrex	41.11	0.1 *N* NaOH
Blank solution ashed in Vitreosil	41.11	0.1 *N* NaOH
Blank solution un-ashed	41.06	0.1 *N* NaOH
Difference	.05	
Equivalent acidity in pounds $CaCO_3$ per ton due to solvent action amounts to—		
For 1 gram sample of fertilizer		1.25 lbs. $CaCO_3$
For 0.5 gram sample of fertilizer		2.5 lbs. $CaCO_3$

COLLABORATIVE RESULTS

The equivalent acidity and basicity values for the six fertilizer samples sent to W. H. Pierre and C. L. Hare are listed in Table 2, along with results obtained by the Associate Referee in a comparative study of two different indicators. The bromophenol blue indicator (0.5 per cent solution) was recommended in last year's report, while the mixed indicator consisting of 0.02 per cent methyl orange and 0.10 per cent bromocresol green was suggested by W. H. Pierre. Ten drops of indicator for 100–150 cc. of solution was used in each case.

The color change of bromophenol blue taken as the end point (*p*H 4.3–4.5) was from yellow magenta to grey or blue magenta, and it occurred just before the definitely blue color at *p*H 4.5. The end point for bromocresol green and methyl orange mixed indicator suggested by Pierre was from slight greenish yellow to a light green, where the green definitely predominated over the yellow.

The values in Table 2 obtained by the writer represent averages of duplicate determinations. The results obtained by titration to *p*H 3.98 represent the values that should be obtained by titration to the first color change in methyl red on the assumption that such change is perceptible as low as this *p*H. (For comparison see *This Journal*, **20**, 264–275 (1937), Table 5.)

In the comparison of the two indicators the average *p*H of the end point of bromophenol blue was 4.37, while that of the mixed indicator (bromocresol green and methyl orange) was 4.55. The results obtained when bromophenol blue was used averaged 3 pounds of calcium carbonate per ton greater basicity than did results obtained with the mixed indicator.

TABLE 2.—*Equivalent acidity and basicity values in pounds $CaCO_3$ per ton compiled by L. E. Horat*

SAMPLE NO.	TULLY	PIERRE	BURTON (HARE)	HORAT-TITRATED WITH BROMOPHENOL BLUE (*p*H)*	HORAT-TITRATED WITH BROMOCRESOL GREEN+M.O. (*p*H)*	HORAT-TITRATED TO *p*H 3.98†
L-3590	137 A	132 A	140 A	135 A (4.32)	141 A (4.52)	95 A
L-3591	112 B	103 B	110 B	118 B (4.34)	110 B (4.44)	145 B
L-3689	330 B	331 B	297 B	345 B (4.24)	337 B (4.58)	362 B
L-3760	332 A	320 A	320 A	338 A (4.46)	343 A (4.58)	303 A
L-3865	340 A	334 A	359 A	323 A (4.44)	331 A (4.56)	261 A
L-4072	360 A	331 A	366 A	325 A (4.46)	337 A (4.62)	235 A

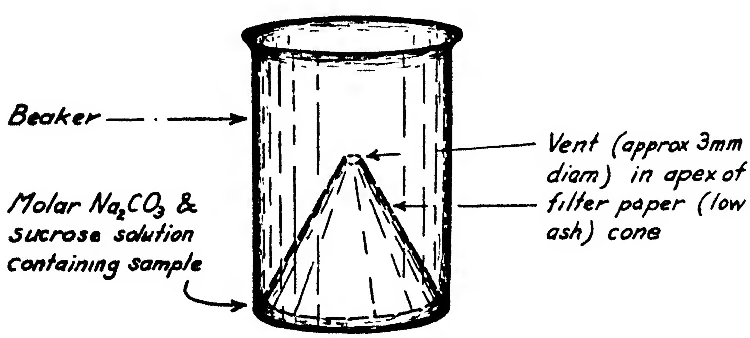
* Determined by means of Beckman *p*H meter after titration.
† Solutions were titrated without indicator to this *p*H.

PREVENTION OF LOSS BY SPATTERING DURING ASHING

Of the 26 collaborators reporting results in 1936, 14 observed spattering during the preliminary drying or ashing of samples.

After trying various means for minimizing this loss by spattering, the Associate Referee found the most efficient method to be the use of a filter paper cone as illustrated in Fig. 5. Provided with a vent of about 3 mm.

Fig. 5 PREVENTION OF SPATTERING DURING EVAPORATION AND ASHING OF SAMPLE



diameter, this cone (of low ash filter paper) is folded so the base will just slip into the beaker, rest on the bottom, and touch the sides all the way around as indicated in the sketch.

The effectiveness of this device may be visibly observed by drying and ashing a sample of mono- or tricalcium phosphate or rock phosphate with and without the filter paper cone. Evaporation may be carried out as rapidly as desired so long as the cone remains in place with the vent open.

Any basicity due to the use of a low ash paper is negligible in this determination.

TABLE 3.—*Acid- and base-forming quality of phosphate rocks* as determined by L. E. Horat*

NO.	SAMPLE	PERCENTAGE OF P_2O_5		NET BASICITY IN POUNDS $CaCO_3$ PER TON
		TOTAL	CITRATE-INSOLUBLE	
L-2812	Tennessee brown rock phosphate	34.8	30.8	262
L-3512	Florida waste pond phosphate	23.3	19.7	364
L-3680	Tennessee brown rock phosphate	31.3	26.3	248
L-3681	Tennessee brown rock phosphate	32.2	28.1	58
L-3682	Tennessee brown rock phosphate	32.0	27.5	154
L-3887	Tennessee brown rock phosphate	32.0	28.1	158
Y-1849	Tennessee blue rock phosphate	10.6	4.9	932

* Determined by tentative method, A.O.A.C., modified to prevent spattering by use of filter paper cone.

BASICITY OF PHOSPHATE ROCKS

In accordance with the recommendation of Subcommittee A on Recommendations, *This Journal,* **20,** 51 (1937), some results obtained in this laboratory are submitted in Table 3. The values represent averages of duplicate determinations made according to the tentative method.

ELIMINATION OF WATER-INSOLUBLE MATERIAL COARSER THAN 20-MESH

In a study of dolomitic limestone supplements of different degrees of fineness, Taylor and Pierre[1] found that all dolomitic limestone should pass through a 20-mesh sieve in order to be available during the year of application. It was also recommended in 1936 that water-insoluble material coarser than 20-mesh be removed by wet sieving before application of the tentative method for the determination of acid- and base-forming quality of fertilizers.

An effort was made in this laboratory to determine the size of sample required in order to get a representative measure of material coarser than 20-mesh by wet sieving. The following study was made:

[1] *J. Am. Soc. Agron.,* **27,** 764 (1935).

Three mixtures (A, B, and C) of fertilizer finer than 20-mesh and dolomitic limestone of 10–20-mesh were so prepared that

A represented 300 pounds dolomite per ton fertilizer;
B represented 150 pounds dolomite per ton fertilizer; and
C represented 60 pounds dolomite per ton fertilizer.

Triplicate samples for each weight of 10, 25, 50, and 100 grams were weighed from each mixture (A, B, and C). Each sample was washed through a 5-inch, 20-mesh sieve with approximately 20 times its weight of water, dried, weighed, and per cent recovery calculated (Table 4).

TABLE 4.—*Recovery of 10–20-mesh dolomite after wet-sieving of fertilizers*

	10 GRAM SAMPLE		25 GRAM SAMPLE		50 GRAM SAMPLE		100 GRAM SAMPLE	
	grams	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*grams*	*per cent*
Mixture A	1.383	92.2	3.442	91.8	6.956	92.7	13.792	91.9
	1.216	81.1	4.109	109.6	6.460	86.1	13.404	89.4
	1.424	94.9	3.353	89.4	6.305	84.0	13.788	91.9
	Av.	89.4	Av.	96.9	Av.	87.6	Av.	91.1
Mixture B	.491	65.5	1.473	78.6	2.386	63.6	6.856	91.4
	.486	64.8	1.261	67.3	3.066	81.8	6.840	91.2
	.568	75.7	1.257	67.0	2.516	67.1	6.874	91.7
	Av.	68.7	Av.	71.0	Av.	70.8	Av.	91.4
Mixture C	.209	69.6	.513	68.4	1.424	94.9	3.345	89.2
	.193	64.3	.623	83.1	1.673	112.0	3.383	90.2
	.208	69.3	.602	80.3	1.442	96.1	3.416	91.1
	Av.	67.8	Av.	77.2	Av.	101.9	Av.	89.8

	Recovered *per cent*
Blanks on 100 grams of 10–20-mesh dolomite	91.4
	90.3
	91.4
	Av. 91.03

The loss in washing 100 grams of samples of 10–20-mesh dolomitic limestone was determined for comparison.

SUMMARY

The experimental evidence presented in the form of titration curves supports previous recommendations that titration in the tentative method can be carried out with less difficulty to an end point of pH 4.3–

4.5. This end point, the least buffered point on the curves, is in agreement with theory and pot tests by Pierre.[1]

Two proposed indicators, bromophenol blue (1% solution) and a mixed indicator (0.10% bromocresol green and 0.02% methyl orange), were compared and found to be satisfactory for titration to the recommended end point.

The solvent action of the molar sodium carbonate solution was found negligible when used as prescribed in the tentative method.

Data submitted in the 1936 report and supported by evidence in this report indicate that the first visible color change of methyl red, as observed in the use of the tentative method, occurs above *p*H 4.3–4.5.

No phosphate rocks investigated were found to be acid by the tentative method.

Preliminary investigation described in this report indicates that at least 100 gram samples of fertilizer should be used in wet sieving for elimination of water-insoluble material coarser than 20-mesh before the tentative method is applied.

The means described for prevention of loss by spattering in the tentative method was found to be completely satisfactory in this laboratory.

RECOMMENDATIONS[2]

It is recommended—

(1) That the present tentative method for the determination of acid- and base-forming quality of fertilizer (p. 34, 55) be modified by the substitution in the titration of 0.5 *N* sodium hydroxide solution in place of 1.0 *N* sodium hydroxide solution.

(2) That further study be given to the same method in regard to changing the indicator from methyl red to bromophenol blue and the use of a filter paper cone to prevent spattering.

(3) That in the same method the elimination of water-insoluble material coarser than 20-mesh before the method is applied be studied further.

(4) That the basicity of phosphate rock and other factors that affect this method be studied further.

ACKNOWLEDGMENT

The writer wishes to express thanks to H. R. Kraybill, W. H. Pierre, C. L. Hare, and all other collaborators for their assistance in this work.

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[1] *J Am. Soc Agron.*, **26**, 278 (1934).
[2] For report of Subcommittee A and action of the Association, see *This Journal*, **21**, 61 (1938).

CONTRIBUTED PAPERS

VITAMIN B_1 ASSAY BY A RAT-CURATIVE* PROCEDURE

By O. L. Kline, C. D. Tolle, and E. M. Nelson (U. S. Food and Drug Administration, Washington D. C.)

The cure of experimentally produced polyneuritis in the rat has been used for a number of years as a measure of vitamin B_1 potency. Heyroth (1) has presented an adequate historical review of the observations dealing with the development of these methods. The technic proposed by M. I. Smith (2) in 1930, with slight modification, has been employed most extensively, and much of the recent quantitative data for Vitamin B_1 has been obtained by this method. Following a similar procedure, Birch and Harris (3) have used bradycardia as a criterion of B_1 deficiency rather than polyneuritis.

In rat-curative methods for quantitatively estimating the B_1, it has been the custom to determine the minimum curative dose for each of a group of animals. Only a few investigators have made an effort to estimate the B_1 in terms of length of cure. The "recovery time" suggested by Hofmeister (4) in 1922 was not adopted by other workers. Van Veen (5), Heyroth (1), and Sebrell and Elvove (6) have supplemented minimum curative dose data with observations of length of cure, while Birch and Harris (3) in measuring cure of bradycardia have observed uniform values for length of curative period.

The writers will describe a method for the estimation of vitamin B_1 by a rat-curative procedure. This method is based on the technic described by Smith (2), but it includes changes in the basal diet and in the method of interpreting the response of the animal to a test dose. With a view to inducing polyneuritis in all the test animals, the basal diet was modified to make it more complete except for vitamin B_1. These diet changes made possible the use of the same test animal for repeated curative tests, and it was demonstrated that the length of the period of curative response in each animal is proportional to the quantity of vitamin B_1 in the test dose administered. The procedure is dependent upon the production of an uncomplicated deficiency syndrome in the test animal.

VITAMIN B COMPLEX

A brief review of the factors included in the vitamin B complex will aid in a discussion of the diet modifications that were found to be necessary. Of these factors (Table 1) the first is vitamin B_1, the antineuritic or anti-beriberi vitamin.

Riboflavin, a yellow fluorescent pigment, is a substance necessary for growth in rats (7) and chicks (8). It prevents and cures alopecia (7) and

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists. November, 1, 2, and 3, 1937.

nutritional cataract (9) in rats and is known to be a component of an oxidation-reduction system in living cells. Riboflavin was synthesized almost simultaneously in the laboratories of Kuhn (10) and of Karrer (11). In German literature this compound is designated as vitamin B_2, while most American workers have described it as vitamin G. In combination with phosphoric acid and a protein carrier it forms the yellow respiratory enzyme of Warburg (12). Little is known at present of the importance of riboflavin in human nutrition. Dann (13) has demonstrated that it is ineffective in curing human pellagra. According to Neuweiler (14) the amount of this factor normally present in human milk is only a fraction of that present in cow's milk.

TABLE 1.—*Vitamin B complex*

Vitamin B_1	—the anti-beriberi or anti-neuritic vitamin.
Riboflavin	—necessary for growth in rats and chicks, prevents alopecia and nutritional cataract in rats, is a component of an oxidation-reduction system in living cells.
Pellagra-Preventive Factor	—a nutritional factor or complex that prevents and cures human pellagra.
Anti-Blacktongue Factor	—prevents and cures blacktongue in dogs—recently reported to be nicotinic acid (amide).
Chick Dermatosis Factor	—prevents and cures a definite deficiency syndrome in chicks.
Vitamin B_6	—prevents and cures nutritional dermatosis in rats (vitamin H, Factor I).
Vitamins B_3 and B_5	—reported necessary for weight maintenance and for rapid weight gains in pigeons.
Vitamin B_4	—prevents a specific nutritional paralysis in rats and chicks.
Factor W	—necessary for growth in rats.

The pellagra preventive (P-P) factor, so designated by Goldberger and co-workers, is a factor or complex that prevents and cures human pellagra. Smith (15) has concluded from work with various sources of the P-P factor that it may be a complex of two or more substances.

The anti-blacktongue factor prevents and cures blacktongue in dogs, a deficiency disease thought to be the analogue of human pellagra. It has recently been reported by Elvehjem and co-workers (16) that nicotinic acid (amide) will cure this condition. Preliminary reports by Smith, Ruffin, and Smith (17); Fouts, Helmer, Lepkovsky, and Jukes (18), and Spies and associates (19) provide definite evidence that nicotinic acid is effective in the treatment of human pellagra.

The chick dermatosis factor prevents and cures a pellagra-like syndrome in chicks (20–21). This substance is present in liver and in yeast. Concentrates prepared from liver from which the vitamin B_1 and ribo-

flavin have been removed (22) have proved effective for the cure of human pellagra, blacktongue and chick dermatosis. It is probable, however, that more than one active substance was present in these concentrates.

Vitamin B_6 prevents and cures a nutritional dermatosis in rats (23). It is probably identical with the substance designated factor Y by Chick and Copping (24), with the vitamin H of Hogan (25) and of Booher (26), and with Factor I recently crystallized by Lepkovsky (27).

Vitamins B_3 and B_5 have been reported as necessary for weight maintenance and for rapid weight gains in pigeons (28–29). These factors in addition to B_1 appear to be necessary for normal pigeon nutrition.

Vitamin B_4 (30) is necessary for prevention of a specific nutritional paralysis in rats and chicks (31–32). Yeast contains very little of this substance; peanuts, wheat germ, and liver are good sources.

Factor W is essential for growth in rats (33). Liver extract is rich in this water-soluble, heat labile substance, which may be precipitated from an aqueous solution by alcohol and ether.

Autoclaved yeast is commonly used in vitamin B_1 studies as the sole source of the vitamin B complex factors other than B_1. With reference to the factors just discussed, autoclaved yeast will furnish riboflavin, the P-P factor, and vitamin B_6. It is, however, low in or devoid of vitamin B_4 and factor W, as the latter is destroyed in the heating process. These two factors may be furnished in supplements exceedingly low in B_1, the B_4 in peanuts that have been autoclaved to destroy B_1, and the factor W in liver extract, which has been purified by alcohol-ether precipitation.

EXPERIMENTAL

In initiating this study the writers were interested in methods of assay of any one of several of the vitamin B-complex factors. It was the purpose to set up a standard diet that might be made suitable for assay of any of these substances by simply alternating the supplements furnishing the necessary water-soluble factors in each case. For this reason highly purified sources of carbohydrate and protein were selected.

Preparation of Diet.—The diet designed for the assay of vitamin B_1 is presented in Table 2. Sucrose serves as a source of carbohydrate. The work of Guerrant, Dutcher, and Brown (34) on sources of carbohydrate for vitamin B studies clearly demonstrates that difficulties due to refection and coprophagy may be minimized if sucrose is used. The casein used is prepared from fresh fluid skim milk by a process involving solution and reprecipitation. The milk is diluted with an equal volume of water, then brought to *p*H 4.6 with dilute (3 per cent) hydrochloric acid. After the casein has settled to the bottom the clear serum is siphoned off. The casein is washed by stirring with a volume of water equal to the original volume of the solution, and the clear supernatant liquid is again siphoned off after the casein has settled. Enough ammonia solution (2.25 cc. of

28% NH_4OH per liter of water) is then added to the casein to bring the volume to that of the original diluted milk. With frequent stirring the casein dissolves (30 minutes or more is usually required). The casein is again precipitated from solution with dilute hydrochloric acid, the serum is siphoned off, and the casein washed. The solution, precipitation, and washing are repeated twice more, and the final precipitate is filtered through cheese cloth, then dried at approximately 60° C. and ground. The salt mixture (Salts I) is described in the U. S. Pharmacopoeia XI under Vitamin A assay. Cod liver oil furnishes vitamins A and D. This part of the diet is regarded as suitable for studies of any of the water-soluble B factors. As more highly purified sources of these factors become available they may be used to replace the crude preparations described below.

TABLE 2.—*Diet for vitamin B_1 assay*

	per cent
Sucrose	61.25
Purified Casein	18.0
Salts I	4.0
Cod Liver Oil	2.0
Autoclaved Yeast	4.0
Autoclaved Peanuts	10.0
Purified Liver Extract	0.75

Autoclaved yeast is prepared by autoclaving dried yeast in layers ¼ inch thick for 5 hours at 15 pounds pressure. It is then dried at approximately 60° C. and finely ground.

Autoclaved peanuts are used as a source of vitamin B_4. Raw, shelled, No. 1 grade peanuts are ground in a food chopper as fine as possible but not to a plastic condition, then autoclaved in layers ¼ inch thick for 5 hours at 15 pounds pressure. This material is then dried at approximately 60° C. For mixing into the diet the autoclaved peanuts may be ground with the sucrose before the other constituents are added.

The liver extract powder used as a source of factor W is essentially the fraction "G" described by Cohn and associates (35). Any liver extract prepared in a similar manner is probably suitable for further purification.* The purified liver extract is prepared by the following procedure, which is similar to that described by Elvehjem and associates (33):

100 grams of liver extract powder is dissolved in 200 cc. of water; 1000 cc. of 95% ethyl alcohol then 1200 cc. of ethyl ether are added with constant stirring. After precipitation is complete (5–15 minutes) the material is filtered through paper, reasonable care being taken to prevent loss of ether. The residue is redissolved in 200 cc. of water, and precipitation and filtration are repeated as directed above. The reprecipitation is repeated a third time, and the final residue may be dissolved in

* NOTE: Only Eli Lilly & Company's preparation 343 was used, but liver extract preparations made by a similar method (See New and Nonofficial Remedies) should be equally satisfactory.

water and added to a small amount of purified casein (200 grams) for drying at approximately 60° C. 100 grams of liver extract will yield approximately 75 grams of the purified preparation.

Depletion of Animals.—Litters of young rats 12–13 days old are placed with their mothers on wire screens in suitable cages, and fed the depletion diet. The young animals are weaned when they have attained a weight of 40–50 grams, which usually occurs at 25–30 days. At the time of weaning the animals are marked and their weights recorded. They are weighed weekly thereafter until the 25th day, when they are segregated on wire screens in individual cages. After the 25th day the animals are weighed daily and observed for symptoms of polyneuritis, which occur 25–50 days after weaning. Up to the present time every animal placed on this diet has exhibited definite symptoms of polyneuritis.

Assay Period.—Polyneuritis may be described as a state of extreme contraction of the musculature, occurring in its early stages after proper excitation of the animal. Excitation is produced by twirling the animal by its tail. Polyneuritis may be divided into three stages: slight, acute, and severe. In the slight stage, recovery from the contraction following excitation is almost immediate. Recovery occurs in the acute stage after several seconds. In the severe stage the recovery period is prolonged, and the animal may show almost continuous uncontrolled movements. Animals in the acute stage are used for the test. Upon the administration of two International Units of vitamin B_1 the symptoms of polyneuritis completely disappear in 24–48 hours, and the animal gains 5–10 grams in weight. This amount of B_1 will prevent the recurrence of polyneuritis for a period of 7–11 days in animals ranging from 40 to 70 grams in weight.

The cure of the syndrome is striking, and recurrence of polyneuritis at the end of the curative period is sharply defined. With some experience it is possible to be consistent in detecting an equal degree of severity of the symptoms at each recurrence of polyneuritis.

It was found that when two International Units of vitamin B_1 were administered at the second occurrence of polyneuritis the animal showed a response similar to the first and the curative period was of equal length. This uniformity of response to a given dose of B_1 was shown by each test animal used, although the length of the curative period varied with, and was thus found to be a characteristc of, each individual.

To determine the range over which an accurate interpretation of potency of dose in terms of length of curative period might be made, the following study was conducted: Each of a series of animals was injected with a dose of 5 micrograms of crystalline B_1 at the first occurrence of polyneuritis, and the period of curative response was noted. These results are shown in Table 3, second column. At the second occurrence of polyneuritis, each animal received an amount of crystalline B_1 indicated in the

third column. The average length of the period of curative response for each of these levels is given in the fourth column of Table 3. Since the variation in response to the reference dose (5 micrograms) is small, the response to doses of different size may be compared directly. The proportion of animals in each group which showed a complete cure is given in the fifth column of this table.

TABLE 3.—*Curative response to different levels of crystalline vitamin B_1*

NO. OF ANIMALS	AV. CURATIVE PERIOD FOR 5 MICROGRAMS OF CRYSTALLINE B_1	AMOUNT OF CRYSTALLINE B_1 ADMINISTERED	AV. CURATIVE PERIOD	TEST ANIMALS COMPLETELY CURED
	days	*micrograms*	*days*	*per cent*
3	—	1.25	0	0
5	9.5	2.5	2.6	60
30	10.0	3	4.0	80
44	9.25	4	5.8	100
12	9.1	5	9.0	100
16	9.8	6	11.7	100
21	9.5	7.5	13.7	100
18	9.1	10	15.8	100
10	8.7	20	21.7	100
6	8.0	50	26.0	100
4	8.8	100	29.0	100

That the response to a given dose is uniformly reproducible is indicated by the group of animals that received 5 micrograms of B_1 in both periods. The average response for 12 animals to 5 micrograms was in the first period 9.1 days, and in the second 9.0 days. The average period for 5 micrograms in 166 of the 169 animals used in the study was 9.0 days, all of which showed a complete cure of polyneuritis and a gain in weight of 5 to 10 grams.

When 1.25 micrograms was administered to each of three animals no cure was obtained. A dose of 2.5 micrograms gave a cure in 3 of 5 animals for an average period of 2.6 days, while the 3 microgram level cured 80 per cent of 30 animals for an average period of 4 days. Not until the 4 microgram level was reached was 100 per cent cure obtained. At this level 44 animals showed a curative response of 5.8 days, compared with an average curative period of 9.25 days with 5 micrograms. The length of the period of curative response increased in proportion to the dose given until the 10 microgram level was reached. This is indicated in Fig. 1, in which length of period is plotted against size of dose administered.

The results obtained with doses of 10–100 micrograms given in a single injection may be explained on the basis of an increased excretion of B_1 into the urine. No attempt was made to determine the amount of the vitamin excreted by these animals. From these results it was concluded

that 7–11 days is the most accurate period of curative response for determining the amount of B_1 administered. Since this period is reproducible and is an individual characteristic of the test animal it allows a comparison of standard and unknown to be made in the same animal.

In these preliminary studies it was possible to use the same animals for a series of tests. In some cases as many as 10–15 curative periods were measured in the same animal without any difficulty. However, the extent to which this repeated use of the test animal may influence the accuracy of the test has not yet been determined.

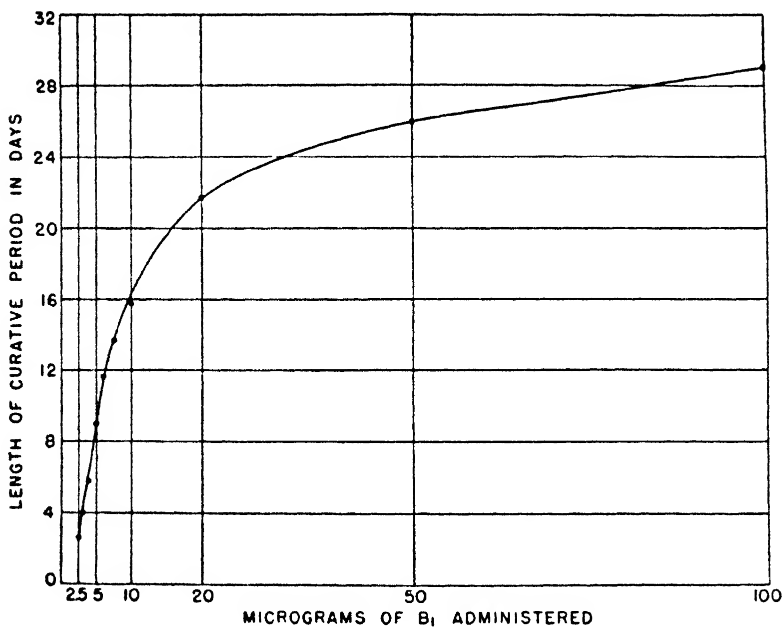

FIG. 1.—CURVE SHOWING THE CURATIVE RESPONSE TO DIFFERENT LEVELS OF CRYSTALLINE VITAMIN B_1

When this technic is used an animal may first be standardized by determining the period of curative response for two International Units of B_1. At the second occurrence of acute polyneuritis a dose of the unknown may be given. If it is desired to check a stated potency, the quantity of the unknown to be fed is, of course, established. If it is desired to determine the exact potency of an unknown, it is preferable to feed it to a few animals at various levels to first determine the approximate quantity that contains two International Units. An assay should then include successive feedings of standard and unknown to at least 8 animals. A given quantity of the unknown contains at least two International Units of B_1 if that quantity promotes in the test animals a total period of curative response (the sum of the number of days of the curative period of each

of the animals) equal to or greater than the total period of curative response for two units of the B_1 standard. This procedure is in contrast to the rat-curative methods described, in which the minimum curative dose for a group of animals over a period of 4 or 5 days is used as an indication of B_1 administered. The procedure presented here eliminates the individual variation by making the comparison in the same animal, and makes use of a test animal showing a sharply defined end point to the curative period.

TABLE 4.—*Vitamin B_1 assay of wheat germ**

ANIMAL NO.	CURATIVE PERIOD FOR 0.3 GRAM WHEAT GERM	CURATIVE PERIOD FOR 2 I.U. VIT. B_1
	days	*days*
178	11	11
191	10	8
209	10	8
228	9	11
235	8	10
237	10	9
242	8	8
247	8	8
	—	—
Total	74	73

* 0 3 gram wheat germ contains 2 I. U. Vitamin B_1.
1 gram wheat germ contains 6.7 I. U. Vitamin B_1.

An example of a typical assay is given in Table 4. In this case the response to 0.3 gram of wheat germ was compared with the response to two units of the International Standard, in 8 animals. The total period for the wheat germ is 74 days, and for the standard 73 days. Thus 0.3 gram wheat germ contains two International Units of B_1, and 1 gram contains 6.7 International Units.

This method is suitable for assay of low potency materials as well as for concentrates of the vitamin. As much as 5 grams of dry material and 10 grams of fresh vegetable material may be fed with reasonable care.

SUMMARY

A method for the assay of vitamin B_1 has been described. It is a modification of the Smith rat-curative technic. A basal diet has been compounded to include recently described essential factors of the vitamin B complex, which when fed to rats produces polyneuritis after a relatively short depletion period. Individual variation is eliminated because a comparison of the curative response of the test animal to standard and unknown is made with the same animal. The length of the period of curative response in animals in which there has been a complete cure is used as a criterion of the amount of vitamin B_1 administered. The method offers

the further economical advantage of conserving test animals needed for a series of assays. The single animal may be used for a number of tests.

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DETERMINATION OF SMALL QUANTITIES OF ANTIMONY IN TARTAR EMETIC SPRAY RESIDUES

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A need for a rapid, simple method for determining small quantities of antimony on citrus leaves developed in connection with experiments carried on by the Department of Agriculture to determine the effect on citrus trees of bait sprays containing tartar emetic, which is an effective poison against certain tropical species of fruit flies.

The method proposed here is based on the reduction by atomic hydrogen of antimony to stibine, which similarly to arsine forms a stain on paper impregnated with mercuric chloride. While arsine forms a characteristic *brown* stain when no other treatment follows, stibine forms a *black* stain when the test paper is subsequently immersed in an alkaline solution.

The method used is a modification of one devised by Shelton, as described by Scott.[1] Instead of paper circles and estimation of the concentration of antimony by the intensity of the stain as proposed by Scott, Hanford-Pratt arsenic strips are used and the concentration of the antimony is estimated by the length of the stain as in the Gutzeit test for arsenic.

The addition of stannous chloride as required by Scott and of potassium iodide as used in the Gutzeit test was found to be unnecessary by tests in which pentavalent antimony was used. In fact, these two substances interfere with the development of the stains.

The soaking of the stained paper in a gold chloride solution, recommended by Scott, was also found unnecessary. This toning may be helpful when permanent stains are desired, but it was found that for quantitative work permanent standard stains are of no value. The character of the stains is so easily affected by various factors that standards must be prepared each time under exactly the same conditions as are used for the unknown values. The known affecting factors are the temperature at which the stibine is generated, the size of the zinc particles,[2] the concentration of the acid in the generator, the concentration of the mercuric salt, and the length of time used for the impregnation of the strips.

When the zinc is too fine (30-mesh or finer) the stains are abnormally pale, have no sharp end point, and are otherwise inconsistent. This is probably due to the fact that the too rapid evolution of hydrogen causes the stibine formed to spread out on the strip too much, and some of it may even escape without reaction with the mercuric salt. In such cases

* Bureau of Chemistry and Soils. Food Research Division Contribution No. 370.
† Bureau of Entomology and Plant Quarantine.
[1] Standard Methods of Chemical Analysis, 4th ed., rev., p. 22. D. Van Nostrand Co.
[2] Cassil, C. C., *This Journal*, 20, 174 (1937).

it was found that the addition of lead acetate to the generator tends to produce normal results. The lead acetate probably acts as a "deactivator," that is it slows up the evolution of hydrogen and stibine.

Quantities of arsenic such as may be found in C. P. reagents do not interfere with the determinations, but appreciable quantities will interfere. However, such quantities can be detected by the formation of the characteristic arsenic stain on the strips before they are immersed in the alkaline solution. In such cases, for the present, it will be necessary to remove the arsenic before the antimony can be determined quantitatively.[1]

The method presented may be used in determining from 25 to 150 micrograms of antimony. The antimony aliquots must be adjusted to fall approximately between these limits. The recovery of tartar emetic from vegetation by this method is simplified because the material may be ashed without loss of antimony. While the method was designed primarily for determining antimony in tartar emetic, the indications are that it may be applied to other forms of antimony as well.

RECOVERY OF ANTIMONY FROM VEGETABLE MATERIAL

While tartar emetic is soluble in water, the recovery of the antimony from the plant surfaces by washing was not feasible because it would be necessary in each case to determine whether the antimony was completely removed. Besides, there is no evidence that soluble antimony will remain soluble after having been in contact with various plant materials and exposed under all weather conditions.

It was found in this investigation that tartar emetic, added to ground air-dried kale, dried orange leaves, and wheat, and ashed in an electric muffle at 550° C., gave stains comparable with those obtained from the same quantities of tartar emetic alone. During the combustion, the antimony added as tartar emetic is apparently oxidized to the pentavalent state. This is indicated by the fact that when sulfuric acid was used in the generator stibine was evolved much more slowly from tartar emetic ashed with vegetable material than from an untreated solution of tartar emetic. Such retardation did not occur when hydrochloric acid was used.

METHOD

Apparatus.—The apparatus used in this investigation was the type specified in the Gutzeit method,[2] with Mill's modification as described by Gross.[3] However, other apparatus built on the same principle may be used. A small Erlenmeyer flask, for instance, may be utilized as a generator, in which case it may be found convenient to use it also for ashing the

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[1] Scott's Standard Methods of Chemical Analysis, 4th ed., rev., pp. 29–32.
[2] *Methods of Analysis, A.O.A.C.*, 1935, 370.
[3] *This Journal*, 18, 189 (1935).

antimony-containing vegetable material. Simple towers filled with small beads or glass wool instead of quartz sand may be used.

Standard Solutions.—A standard stock solution was made by dissolving 0.2734 gram of C. P. antimonyl potassium tartrate $K(SbO)\text{-}C_4H_4O_6 \cdot \frac{1}{2}H_2O$) in distilled water and diluting it to 1 liter; 1 cc. of this solution is equivalent to 100 micrograms of antimony (Sb). The quantities 25, 50, 100, and 150 micrograms used in these experiments were obtained by taking aliquot parts of a tenfold dilution. Both the stronger and the weaker solutions became moldy after about 2 weeks and were not used in this condition.

Procedure.—One gram of vegetable material to which known concentrations of antimony had been added was ashed in a porcelain dish in an electric furnace at 550° C. The ash was carefully transferred to a generator, and the dish was washed with a known volume of concentrated hydrochloric acid not in excess of 5 cc. Enough hydrochloric acid was added to make 5 cc., and the volume was made up with water to 40 cc. The solutions were allowed to cool to room temperature. The towers were saturated with a 10 per cent lead acetate solution.

The Hanford-Pratt test strips were soaked for 2 hours in an aqueous 4 per cent mercuric chloride solution. The strips were air-dried, and one was inserted in each of the small tubes above the towers. The small tubes must be thoroughly dried before the strips are inserted. Four or five drops of a 10 per cent solution of lead acetate were added to each generator and finally 2 grams of 30-mesh (or coarser) zinc. The generators, tightly connected with the towers by means of rubber stoppers, were placed in a water bath at 20–22° C., and the reaction was allowed to proceed for not less than 1.5 hours. However, except in very hot summer days, the tests may be run at room temperature.

On removal from the generators, the strips were immersed in 5 per cent ammonia solution (180 cc. of concentrated ammonium hydroxide made up to 1 liter with distilled water) for about 2 minutes and washed first with water and then with alcohol. The strips were dried, and the stains on both sides were either measured immediately or marked with a pencil and measured when convenient.

RESULTS

The work in this investigation was carried out with solutions of tartar emetic of known concentrations. Typical results obtained in seven experiments are given in the table. The figures in all cases are averages of duplicate determinations and represent the sums of the measurements of the stains on both sides of the strip. The agreement between duplicates in most of the cases was fair.

Under the headings marked "standard" are given the results obtained with untreated solutions of tartar emetic. They show that the length of

Recovery of antimony added to ground orange leaves and subsequently ashed

ADDED ANTIMONY (MICROGRAMS)	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3			EXPERIMENT 4			EXPERIMENT 5			EXPERIMENT 6			EXPERIMENT 7		
	LENGTH OF STAINS			LENGTH OF STAINS			LENGTH OF STAINS			LENGTH OF STAINS			LENGTH OF STAINS			LENGTH OF STAINS			LENGTH OF STAINS		
	STANDARD	ORANGE LEAVES	RECOVERY	STANDARD	ORANGE LEAVES	RECOVERY	STANDARD	ORANGE LEAVES	RECOVERY	STANDARD	ORANGE LEAVES	RECOVERY	STANDARD	ORANGE LEAVES	RECOVERY	STANDARD	ORANGE LEAVES	RECOVERY	STANDARD	ORANGE LEAVES	RECOVERY
	mm.	mm.	per cent	mm.	mm.	per cent	mm.	mm.	per cent	mm	mm.	per cent	mm.	mm.	per cent	mm.	mm.	per cent	mm.	mm.	per cent
25		22.0	110		21.0	70		20	90		19.5	90	18.0	17.0	90	21.5	17.5	80		18	80
40																	25.5	75			
50	38.5	37.5	97	52.0	47.5	90	43.0	40.0	94	39.0	39.5	100	36.0			40.5			40	40	100
55														38.5	100						
60																	39.0	79			
100	70.5	70	100	69.0	73.5	115	80.5	72.0	87	72.5	80.5	115	58.0			65.0	67.5	106	70	66.0	93
110														62.5	95						
150	101			81.0	85.0	110	98	97	97	92.5	96.0	108	84.0	77.0	91	82.0	78.5	93	90	92	105

the stains may vary considerably for the same concentration from experiment to experiment. They also show that the length of the stains in a single experiment is not strictly proportional to the quantity of antimony, the larger quantities giving proportionately shorter stains than the smaller quantities.

The results given under the headings "orange leaves" were obtained with known concentrations of tartar emetic added to ground orange leaves and subsequently ashed in an electric furnace at 550° C. They were considered as unknown quantities, and their recovered values were determined from the position of their stain-lengths on the standard curve prepared as directed in the Gutzeit procedure. The percentages of recovery were obtained by comparing these recovered values with the actual quantities of antimony added to the orange leaves. The results indicate that there is no loss of antimony during ashing, as the recovery fluctuates from below to over 100 per cent, the general average of recoveries being 95 per cent. Only in one case was the recovery as low as 70 per cent.

SUMMARY

The stain method for the determination of antimony has been modified and simplified with reference to both apparatus and procedure. When known concentrations of tartar emetic were used it was found: (a) that when lead ions are present in the generator better and more consistent stains are obtained; (b) that pentavalent antimony may be transformed into stibine without being previously reduced to the trivalent state; and (c) that antimony from tartar emetic may be recovered from vegetable material ashed at 550° C.

ANALYTICAL METHODS APPLICABLE TO BEER*

By E. Singruen (Modern Brewer, 205 E. 42nd Street, New York City)

Among the numerous analytical methods applicable to beer suggested in the recent brewing literature two types of methods in particular have attracted considerable attention and promise to become of great practical value, namely, the determination of the oxidation-reduction potential and, closely connected with this factor, the determination of air in beer.

In 1934, Jean de Clerck (4) in Belgium first applied rH measurements to the study of beer stability, chiefly with respect to the influence of oxidation by contact with air on its keeping qualities. Among others Mendlik (17) and Van Laer (25) in Europe and Laufer (14) in New York took up this phase of investigation, and the results published so far appear to justify the expectation that these methods may be useful in the explanation of many oxidation and reduction reactions.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washton, D. C., November 1, 2, and 3, 1937.

The results available seem to show that beer possesses a rather strong, reversible redox system, which has the tendency of bringing the rH to an equilibrium at 17–18. Subsequently, another weaker system becomes active, reducing the rH to 10–12, if no excess air is present. At the present time, however, it is not known which of the constituents of beer is responsible for its reducing power.

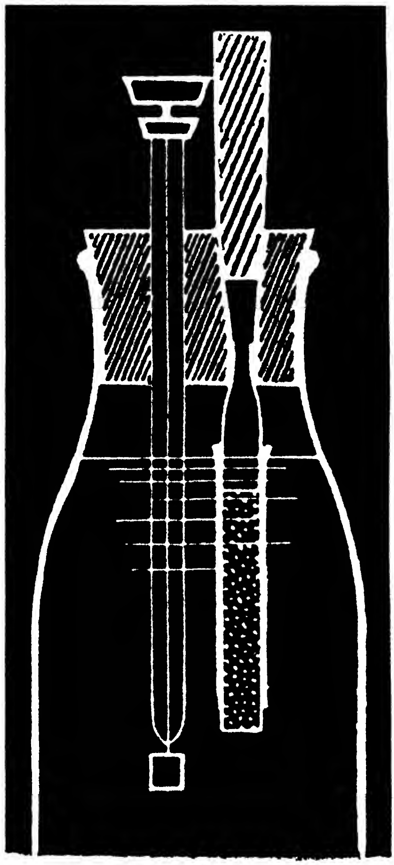

FIG. 1.—DE CLERCK'S ARRANGEMENT FOR MEASURING THE rH REACTION WITHIN THE BOTTLE

Important conclusions of practical value have already been derived from this more or less preliminary work. The keeping quality of beer can be greatly improved by preventing the beer from coming in contact with air after fermentation, particularly during the filling operations.

It has been demonstrated that up to 0.5 ml. of oxygen per liter of beer can be tolerated without harmful effects on its stability. If present in larger quantities in packaged beer, the air stimulates the formation of

yeast and of colloidal and pasteurization turbidities and produces or increases the so-called "light-taste," which has been found to be the result of a reduction.

The most accurate and therefore standard procedure of determining the rH, of course, is the electrometric method, whereas colorimetric procedures lend themselves with greater advantage to the practical routine control of aeration in beer.

Recently deClerck (4) developed a very simple electrometric method by means of which the rH can be measured in bottled beers, thus permitting a more thorough study of the oxidation-reduction reactions taking place within the bottle. The bottle is closed with a two-holed rubber stopper carrying on one side a brilliant platinum electrode and

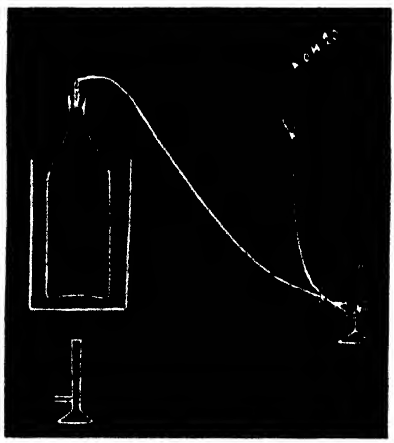

FIG. 2.—DE CLERCK'S SET UP FOR THE AIR DETERMINATION IN BOTTLED BEER

on the other, a connecting siphon. In order to prevent the beer from escaping through the siphon, a small porous cylinder, similar in shape to the cylinders used for establishing the liquid connections between certain quinhydrone electrodes, is attached to the lower end of the siphon. The tube itself, filled with saturated potassium chloride, reaches to the center of the stopper and is closed with a glass rod. In order to measure the potential, this rod is replaced by a saturated potassium chloride siphon connected with a calomel electrode, and the difference between the potentials of both electrodes is determined.

Inasmuch as rubber is not completely impermeable with respect to carbon dioxide under the pressure that develops in the bottle during heating, the bottle is placed in a horizontal position for the duration of the test, so that the beer covers the lower surface of the stopper.

For routine tests simple, practical colorimetric methods have been developed for the control of aeration. Suitable indicators are added to the

beer, and the rH is determined by observing the color changes in the bottle. Since the indicator itself is a redox system, only minute quantities are permissible. A dilution of 1 part of dye to 500,000 parts of beer is sufficient for light beers. The results thus obtained have been found to be practically identical with those obtained by electrometric measurements.

deClerck developed the following scheme to test the usefulness of various indicators in beer analysis:

Indicator	*rH of change*	*rH of beer*
Phenol-indo	22	
2:6 dibromophenol	20	
o-cresol-indophenol	20.5	
	18.5	
1 Naphthol-2-sodium	18.5	
sulfonate indophenol	16.5	at bottling
Cresol blue and	17.0	
Lauth's violet	15.0	
Methylene blue	15.5	reduction in bottle
	13.5	
Indigo tetrasulfonate	12.0	
	11.0	
Indigo trisulfonate	11.5	final rH
	9.5	
Indigo disulfonate	10.0	
	8.0	

Laufer (14) also published the results of a comprehensive study of the suitability of various indicators.

Methylene blue has been used extensively by most investigators, because it is reduced at an rH (13.5–15.5) suitable for this purpose and because it produces distinct color changes in beer.

QUANTITATIVE DETERMINATION OF AIR IN BEER

Measurements of rH, however, provide only qualitative results. With the recognition of the effect of air on beer, methods were demanded by which the air content of beer could be determined quantitatively. A number of procedures were developed and reported in the literature.

deClerck (4) proposed a method according to which the bottle is opened under mercury and re-closed by a rubber stopper carrying a bent glass tube to which a thin rubber tube is attached. The air-carbon dioxide mixture in the bottle neck is forced into an eudiometer filled with 20 per cent potassium hydroxide. The carbon dioxide is absorbed, and the amount of air can be read in a calibrated glass tube. In order to determine the quantity of air that is dissolved in the beer, the sample is heated in

a water bath. The percentage of oxygen in the air can be determined by adding 10 per cent sodium hydrogen sulfite to the potash, filling the eudiometer with water, and mixing its contents until the gas volume remains constant. The difference between the first and second readings represents the oxygen. This latter procedure gives only correct results for the air in the bottle neck. The oxygen in the dissolved air partly enters into reactions with other beer constituents during heating. de-

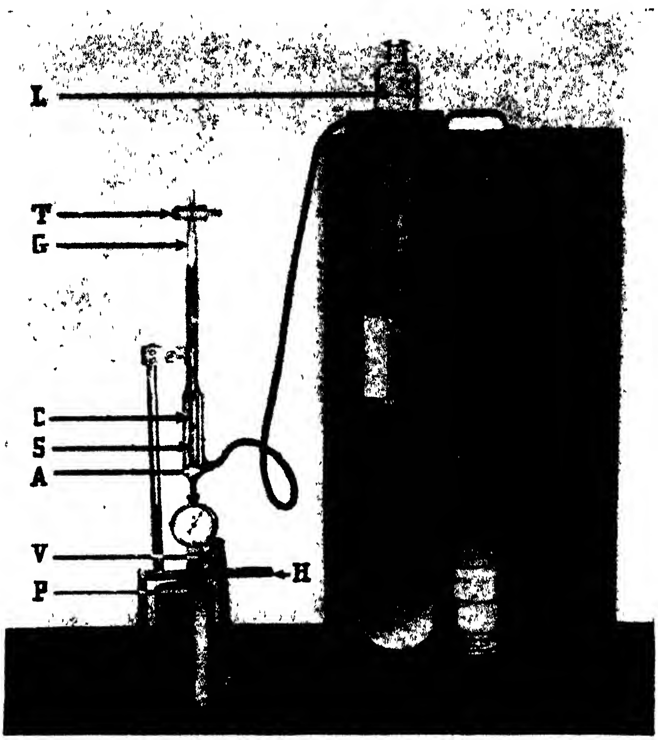

Fig. 3.—Baselt's apparatus for the determination of air in packaged beer.

Clerck reports that in 1 liter of beer containing 1.6 ml. of dissolved oxygen, 0.45 ml. of the oxygen was lost during heating.

Baselt (1) described a compact, portable apparatus and a procedure for the determination of air in both bottled and canned beer. The test is carried out at 77° F. The bottom of an absorption chamber is connected with a needle valve and a piercing device. The carbon dioxide is absorbed in strong caustic soda, and the gas not absorbed and read in a calibrated glass tube is taken as air. This method is particularly suitable where a long series of samples has to be tested within a short period of time. It is also applicable to other carbonated beverages.

Siegfried (18), in Switzerland, suggested a procedure that involves the transfer of the beer by carbon dioxide pressure from a bomb into an air-free, carbon dioxide filled bottle, which is heated to boiling in a carbon dioxide stream. The escaping air-carbon dioxide mixture is measured. After the carbon dioxide has been absorbed and the air contained in the carbon dioxide of the bomb has been subtracted the remaining volume of gas represents the air content of the beer.

Helm (11), in Denmark, advanced three methods for determining the air content of beer.

COLOR AND TURBIDITY MEASUREMENTS

Another field of brewing chemistry in which physico-chemical methods have been applied includes color determinations and turbidity measurements.

In an effort to put the determination of color in beer, wort, and malt on a more scientific basis, Enders and his associates (15, 16) investigated the value of absorption measurements. It was shown that it is actually possible to express the color of beers accurately by their absorption values by comparing them with standard absorption curves of standard melanoidine solutions.

Inaccuracies encountered in turbid beers caused by the interference of colloids can be reduced to a minimum by using highly filtered light according to Krumholz and collaborators (13).

If the absorption of turbid beers is determined in red light, even high turbidities can be ignored. Up to comparatively high values the extinction in blue is generally twice that in red, therefore extinction in blue minus twice the extinction in red is sufficiently accurate for color in turbid worts and beers. Various instruments, such as the Lange colorimeter and a microphotometer with light filters, have been tested for their suitability, the latter being standardized directly by means of dilution series of beer and malt worts.

Based on either the absorption of transmitted light or on the close relationship between extinction in red light and turbidity, studies are being made on measuring the degree of turbidity in colored liquids by means of the same instruments. After the absorption due to the color is allowed for, the turbidity is measured by absorption and comparison with the curves of standard solutions. The degree of turbidity is expressed in mg. of a turbidity-forming substance of similar physical character, mastic being used for colloidal turbidities and silica gel for suspensions.

deClerck is of the opinion that the Zeiss-Pulfrich photometer gives the most satisfactory results. With this instrument the influence of the light absorbed by color is negligible in light beers up to 15° Lovibond; in dark beers compensation must be made for this factor. The Lange colorimeter is also being used for measuring the rate of sedimentation.

During the turbidity measurement it was observed that time changes of per cent absorption in suspensions can be applied to sedimentation measurements. Although a direct determination of the degree of dispersion is impossible with this instrument, inasmuch as there exists no definite relationship between absorption of light on one side and number and size of particles in suspension and speed of sedimentation on the other, a purely comparative evaluation by means of sedimentation curves is usually sufficient for technical purposes. This procedure is still practicable in cases where the difference between the specific gravity of the dispensed material and the dispersion medium is so small that kinetic methods fail to give results.

ELECTROMETRIC TITRATION

A simplified electrometric titration method has been applied successfully to the determination of acidity in beers by Jukes (12) in England. A buffer solution of the same *p*H as that of the desired titration end point with the addition of a little quinhydrone is used as a reference half-cell. It is connected by a potassium chloride bridge to the solution to be titrated, also containing quinhydrone. The electrodes dipping into each of the half-cells are connected with a sensitive galvanometer and tapping key. The acid solution is titrated with alkali until the galvanometer shows no reflection, thus indicating the end point.

Salac (20) made use of the polarograph in the determination of cystin in beers. The differences between the cystin content of different brewing liquids were distinct and corresponded to the amino acid content as determined by the formol titration. This instrument may become an important tool in the study of oxidation-reduction reactions in the cystein-cystin system, which is reversible.

Three other methods deserve to be mentioned as being applicable to beer.

Walters (26) described a modification of the micro-colorimetric method proposed by Fiske and Subbarrow (9) for the determination of the total phosphoric acid in barley, malt, yeast, wort, and beer and of the inorganic phosphates in the two latter liquids.

Schild and collaborators (21, 22) reported a procedure that they found reliable for the determination of tryptophan in wort and beer. Preliminary tests with this method indicated that only that portion of the tryptophan is determined which is present as free amino acid or which forms the final link of a peptide. The method by Folin and Marenzi (10), based on the reduction of the phenol reagent by tryptophan, is not applicable to wort and beer. All values obtained by this procedure are too high because of simultaneous reactions with other constituents, such as melanoidines.

Finally, there is an analytical procedure by Sichert and Bleyer (19), by means of which glucose, maltose, and dextrin can be determined in

the presence of all three carbohydrates in sugar mixtures. It consists of:

(a) Determination of glucose by reduction of copper sulfate-sodium acetate solution;

(b) Determination of glucose and maltose together by reduction with Fehling solution; and

(c) Determination of the total sugars as glucose by reduction of the inverted sugar solution by means of Fehling solution.

These authors also discuss the advantages of the Steinhoff copper sodium acetate solution over Barfoed's reagent (acetic acid-copper acetate solution) in the determination of dextrose. The iodometric titration of the cuprous oxide, suggested by Steinhoff, however, is preferred to the titration with potassium permanganate.

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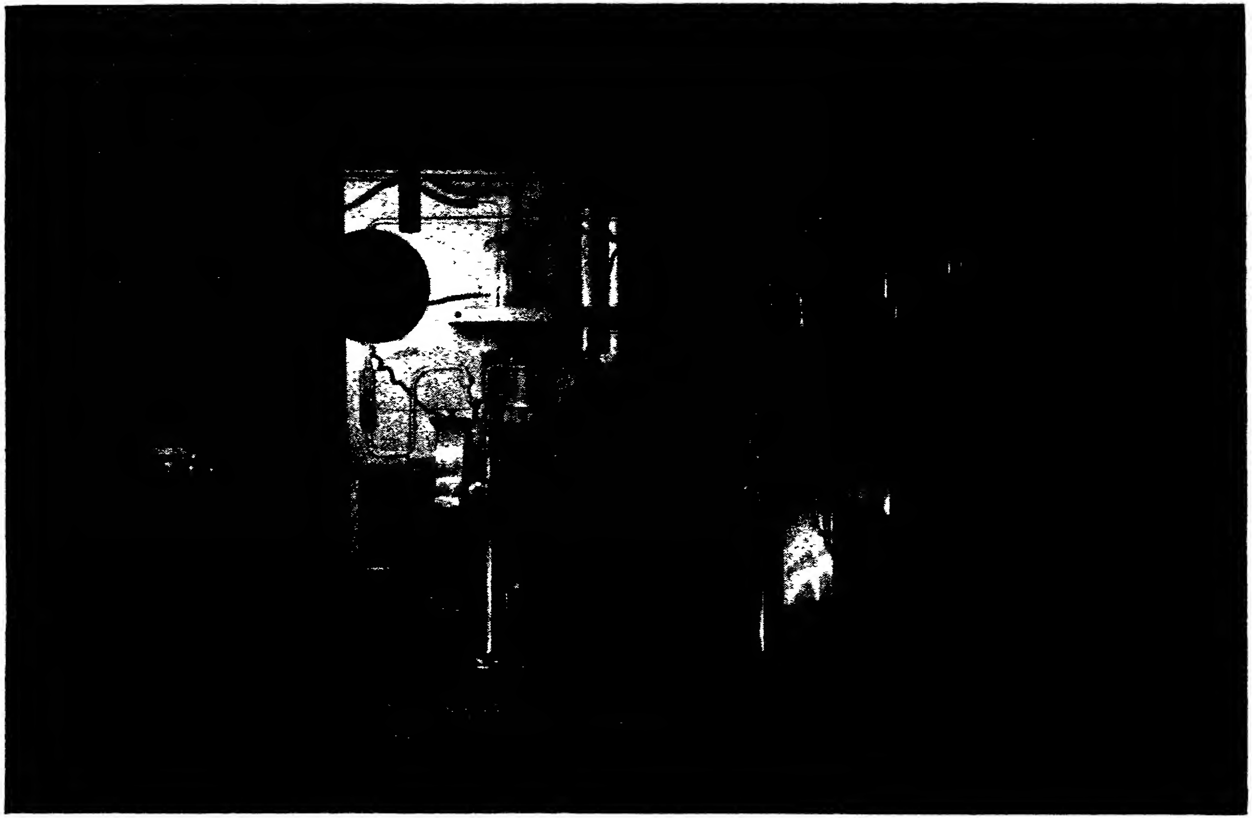


General View of Ozonizer

AN OZONIZER FOR LABORATORY USE*

By M. J. Goss and Max Phillips

The ozonizer described in this paper is essentially a modification of the apparatus described by Briner, Patry, and de Luserna[1] and by Smith.[2] It is compact, safe, and easy to operate. The apparatus has been in use in this laboratory for several years and has proved to be entirely satisfactory. A description of it is presented here in the hope that it may prove to be of value to others who are required to conduct ozonization experiments.

GENERAL DESCRIPTION

Ozonizing Tubes.—The ozonization of the oxygen is effected in the three Berthelot tubes (Fig. 1 and photograph), L, M, and N, which are connected in series by means of mercury seals. Each Berthelot tube (see detail sketch of ozonizing tube) consists of an outside tube, C, 41 mm. in diameter and 48 cm. long, provided with an inlet tube, Z' and an exit tube, Y'. Into tube C a second tube, X', 35 mm. in diameter and 45 cm. long, is so sealed that the annular space between the two tubes is always 3 mm. across. Tube X' is supplied with a glass tube B, 25 mm. in diameter and about 52 cm. long. Tube B is closed at one end, and a small piece of rather heavy platinum wire is sealed through the bottom. Tube Z' is provided with an "outside seal" and exit tube Y' with an "inside seal." Each ozonizing tube is supported on a cork base, D, which is suitably carved out, so that it fits well around C without producing any strain on Z'.

Cooling Coil.—The cooling coil, D'-C', consists of a $\frac{3}{8}$ inch copper tubing and is bent in the form of a flat coil having the general shape shown in the top view of the drawing. The coil is held in place by projecting the ends C' and D' through the wooden top G'.

Battery Jar.—The battery jar, F', is $8\frac{1}{2} \times 12\frac{3}{8} \times 17$ inches high. It is provided with a wooden top, G', through which the ozonizing tubes and the ends of the cooling coil project. The top is made of several wooden parts, which are held together with wooden strips and screws.

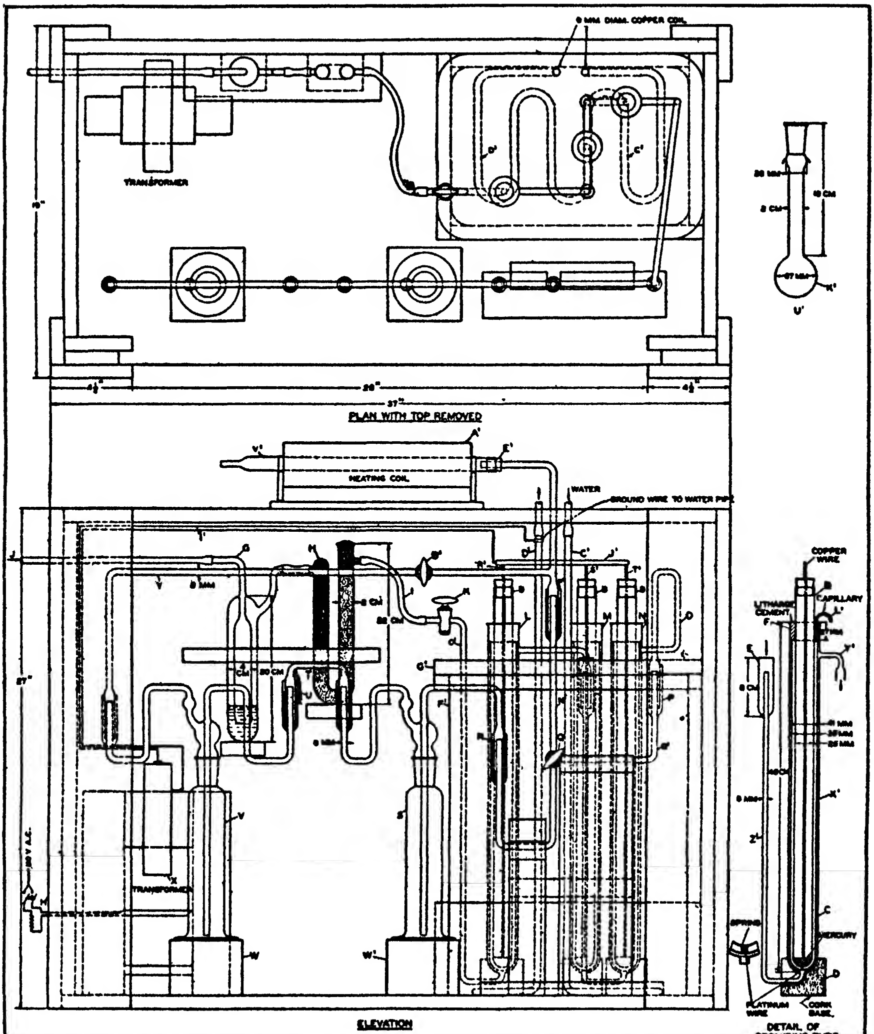
Ozone Destroyer.—The ozone destroyer consists of an electrically heated furnace, A', into which is inserted glass tube V', which is filled with granular (about the size of peas) manganese dioxide. The glass tube delivering the ozonized oxygen is attached to V' by means of a stopper, E', made of "transite." The furnace is provided with a rheostat not shown in the drawing. The position of the sliding contact of this rheostat is determined in order to get the desired temperature. This is done by removing V', placing a thermometer in the furnace, and determining the position of

* Contribution No. 285 from the Industrial Farm Products Research Division, Bureau of Chemistry and Soils, U.S. Department of Agriculture.

[1] *Helv. Chim. Acta*, 7, 62 (1924).

[2] *J. Am. Chem. Soc.*, 47, 1844 (1925).

the sliding contact when the temperature of the furnace is approximately 250° C. As the temperature need not be maintained exactly at 250° C., this method of control is sufficiently accurate for the purpose. For convenience, the ozone destroyer is fastened to the top of the cabinet.



OZONIZER

Drying Train.—The oxygen from a cylinder provided with a reducing valve is led through rubber tube *J*, and through the sulfuric acid wash bottle *G* and drying tube *H*. Approximately one-third of the *U* tube is filled with "ascarite," and the remaining space is filled with calcium chloride. It is well to paint with hot paraffin the rubber tubes *J* and *I*

and the rubber tube connecting *G* and *H*. This helps to preserve them. Ozone destroys rubber tubing very rapidly.

Reaction Vessels.—The solution containing the substance to be ozonized is placed in *S*, which is provided with a No. 20 interchangeable ground-glass joint. *S* is supported by wooden block *W'*. When it is desired to cool the reaction mixture during ozonization, it is more convenient to use flask *U'*. This is also provided with a No. 20 interchangeable ground-glass joint and may, therefore, readily replace *S*. When in use, *U'* is supported with a clamp attached to a ring stand and is cooled with ice or by means of some other cooling medium.

Some of the solvent used for carrying out the ozonization is placed into *V*. This serves merely for the purpose of observing the rate at which the gas passes after leaving the reaction vessel. *V* is connected to either *S* or *U'*, as the case may be, by means of tube *T* inserted into the two outer mercury seals. *T* is held in place by means of bronze spring *U*.

Glass Tubing.—The apparatus is constructed from Pyrex tubing. However, tubes *B* of the three ozonizing tubes, *L*, *M*, and *N*, are made from soft glass tubing.

Transformer.—A high voltage transformer is used. The primary current of 110 volts, 60 cycles, is increased to 25,000 volts on the secondary.

Cabinet.—The cabinet for housing the apparatus is 37 inches long, 19 inches wide, and 27 inches high. The front of the cabinet is provided with a sliding glass (preferably non-shattering) door, 29½ inches long and 26 inches high. This cabinet is for the protection of the operator as well as of the apparatus. The housing of the entire apparatus in this cabinet facilitates the moving of the ozonizer when occasion arises. The photograph shows a general view of the assembly.

ASSEMBLING THE APPARATUS

Ozonizing Tubes.—Each double-walled Berthelot tube (*C* sealed to *X'*) is fastened to a ring stand and into each tube is inserted a small spring of steel wire (about 1.5 cm. long). Tube *B* is lowered into place, while care is taken to see that the piece of platinum wire emerging from the bottom of tube *B* is encircled by the steel spring, since the spring serves to prevent the puncturing of tube *X'* by the wire. The tube *B* is carefully centered so that the annular space between *X'* and *B* is as uniform as possible, and *B* is then securely fastened to the ring stand. The annular space between *B* and *X'* is filled with mercury. The top of the mercury column is adjusted to about 27 mm. from the top of tube *C*. A small capillary tube, *L'*, bent in the form of a hook and reaching down to the mercury, is put into place, and the space remaining between the tubes is filled with litharge-glycerol cement. The three assembled ozonizing tubes are allowed to stand for several hours, or until the cement has completely hardened.

Into *B* of each of the three ozonizing tubes a few cubic centimeters of mercury is poured.

Ozonizer.—The three ozonizing tubes (*L, M*, and *N*) are placed in the battery jar *F′* and supported on their cork bases. The tubes are held in place by hand while an assistant marks on the bottom of the battery jar the exact positions of the three cork bases. The tubes are then removed, the bottom of each cork base is smeared with warm pitch, and the corks are replaced in their respective positions on the bottom of the battery jar and allowed to cool. The cooling coil is placed and then the three ozonizing tubes are put in the jar. The battery jar is filled with distilled water, and the several parts of the wooden top *G′* are then fitted around *L, M, N, D′*, and *C′*, as well as around the top of the battery jar and are fastened together by means of wooden strips and screws. The battery jar is placed in the cabinet and is held securely by means of two boards, about 5 inches wide, which are fastened to the bottom and sides of the cabinet. Two rubber tubes are passed through two holes in the back of the cabinet and the tubes are attached to the cooling coil *C′* and *D′*. Water is circulated through the copper coil in the manner shown in the drawing. Tube *Q′* with outer seals, *P, P′*, and *R* and the three-way stopcock *Q* is connected to *O* and then supported in place by means of a wooden holder placed between *Q* and *Q′*. Connections between *R, S, T, V*, and *Y* are then made in the manner shown in the drawing. *G* and *H* are held in a wooden holder, which is attached to the back of the cabinet. They are connected with a small rubber tube, and *G* is connected by means of rubber tube *J* to a cylinder of compressed oxygen provided with a reducing valve. Rubber tube *I* is connected to glass tube *O′*, which is provided with a glass stopcock, *K*. Tube *Y* is fastened to the ceiling of the cabinet, and mercury is put in all the outer seals.

Electrical Connections.—The two electric wires are attached to the two poles of the primary coil of the transformer. An electric switch and a rheostat are placed in this line. One of the poles of the secondary coil is connected to *D′*, while the second pole is connected to brass rod *J′*, to which are soldered the three copper wires, *R′, S′*, and *T′*. As shown in the drawing these wires pass through corks into tubes *B* of the three ozonizing tubes. *R′, S′*, and *T′* make contact with the mercury in each of these three tubes. *D′* is grounded to a water pipe, and the other wire, attached to *D′*, is placed in such a manner that the danger of contact with the wire connected to *J′* is minimized. An electric light is fixed inside the cabinet, and it may conveniently be connected to the electric circuit of the heating coil *A′*.

Operation.—*A′* is first heated to the desired temperature. Stopcock *K* is opened and a slow stream of oxygen is passed through *G, H*, and the three ozonizing tubes and then through *Q′, S, T, V*, and *Y*. The switch connecting the transformer with the 110 A.C. line is pushed in, and the

ozonization of the sample is allowed to proceed until the reaction is complete. By means of the three-way stopcock Q and the stopcock B' the ozonized oxygen may be passed either up through N' and then directly into the ozone destroyer or it may be passed through the solution in S, then through T, V, Y, and B' into the ozone destroyer. Stopcock K is always kept closed when the apparatus is not in use, otherwise the residual ozone will disintegrate rubber tube I.

APPLICATION OF THE NEUTRAL WEDGE PHOTOMETER TO THE MEASUREMENT OF CAROTENOID PIGMENTS IN FLOUR AND MACARONI PRODUCTS*

By VIRDELL E. MUNSEY (Food Division,† U. S. Food and Drug Administration, Washington, D. C.)

The yellow carotenoid pigment of flour has been generally recognized as carotene. The availability of an accurate, relatively simple method for its estimation should be helpful in advancing studies on the composition of cereals and flours. The knowledge gained through the application of such a method is fundamental in the control of the use of carotene in food products for purposes of coloring, such as recent proposals for its addition to macaroni products.

MEASUREMENT OF THE CAROTENOID PIGMENT IN FLOUR

Winton (1) (1911) extracted the yellow color from flour with colorless gasoline and compared it with an 0.005 per cent potassium chromate solution in a colorimeter in order to differentiate bleached and unbleached flour. Many efforts have been made to improve the Winton method, and special emphasis has been given to the search for a reference standard having the same hue as the flour extract. Jorgensen (2) modified Winton's standard by buffering the chromate solution to a definite pH. Kent-Jones and Herd (3) used a mixture of potassium chromate and cobalt nitrate. The various other standards used include alizarin in chloroform, napthol yellow and orange G in water, and azobenzene in alcohol. It is generally recognized that none of these reference standards is completely satisfactory.

Ferrari and Bailey (4) (1929) published a quantitative method for the determination of carotenoids in flour, calculated as carotene, in which the pigment is extracted with a mixture of 93 per cent naphtha and 7 per cent absolute alcohol. This procedure is based on the work of Schertz (5), who measured the amount of carotene in solution by means of the spec-

* From a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1937.
† W. B. White, Chief.

trophotometer. The principal disadvantage of the method seems to be the use of this instrument, which is expensive and not generally available.

Geddes (6) et al (1934) compared the spectral distribution curve of flour extract with the Winton potassium chromate, the Kent-Jones chromate-cobalt nitrate, and the Sprague naphthol yellow-orange G standards and found the curves to cross at the mercury arc line, 4358A°. They concluded that all three standards are satisfactory from the standpoint of identity of spectral characteristics at this line, but on the basis of reproducibility and permanence they chose potassium chromate as their standard. The chromate solution, previously standardized against carotene, is matched against the flour extract, a mercury arc being used as a source of light. The results on flour extracts are expressed in parts per million of carotenoid pigments as carotene.

Ferrari and Croze (7) (1934) report that a flour extract prepared as directed by Ferrari and Bailey showed no significant change, as determined by the spectrophotometer, when stored 291 days in a brown, glass-stoppered bottle. They proposed that a standard flour extract be used as the reference in making comparisons in the colorimeter or Nessler tubes. A spectrophotometer would, of course, be necessary to standardize the reference flour extract at definite intervals.

CHARACTERIZATION OF THE CAROTENOID PIGMENTS OF FLOUR

Monier-Williams (8) (1912) reported that carotene was the pigment of wheat flour. Ferrari and Bailey (4) (1929) compared the absorption spectra of a preparation of pure carotene with flour extracts and reported that "the spectral distribution curve for the flour extract possessed carotin characteristics, but evidence was obtained of the presence of another pigment in addition to carotin." Their evidence tends to establish the pigment of flour extract as carotene with little, if any, xanthophyll. Markley and Bailey (9) (1935) used the Willstätter and Stoll (10) method of separating carotene from xanthophyll, which is based on the preferential solubility in an immiscible naphtha-methanol mixture. Their analyses of four samples of wheat showed 12.8–34.8 per cent of the total pigment to be carotene. They also determined absorption curves on the naphtha (carotene) and methanol (xanthophyll) fractions, but they did not draw any definite conclusion as to the nature of the pigments.

Both Ferrari and Markley attempted to characterize the carotenoid pigment in flour by chromatographic analysis on the theory that xanthophyll is adsorbed on calcium carbonate from a hydrocarbon solvent. Ferrari and Bailey (4) failed to get adsorption of a flour extract on a calcium carbonate tower, and Markley and Bailey (9) found part of the flour pigment was adsorbed in passing through a 42 inch calcium carbonate tower.

GENERAL DISCUSSION

The laws of Lambert (11) and of Beer (12) play an important role in colorimetry, photometry, and spectrophotometry. Combination of these two laws gives the fundamental equation of colorimetry and spectrophotometry,

$$E = -\frac{1}{CL} \log T,$$

or that used for calculation of concentration in the form,

$$C = -\frac{1}{EL} \log T.$$

The value of E depends upon the manner of expressing the concentration. If C is expressed as molar concentration, E denotes a molar extinction coefficient. In this present study, C is expressed in milligrams per liter; L represents cell depth of solution; E extinction coefficient; T, transmittancy,* which is the ratio of the transmission through solution and solvent; and $-\log T$ equals absorbancy.

The neutral wedge photometer used in this work is the one described by Clifford and Wichmann (13), modified by the designers by the substitution of a permanent glass wedge for the gelatin wedge and improvement of the light source.

PROPERTIES OF CAROTENOID PIGMENTS (15) (18–20) (21)(24–26)

A knowledge of the properties of the carotenoid pigments is essential, especially for the characterization of the pigments in flour and the development of a method for their extraction and measurement. The two principal carotenoid pigments concerned in this study are carotene and xanthophyll, although kryptoxanthine occurs in noodles due to the egg present. All these pigments have molecules with 40 carbon atoms, 56 hydrogen atoms, and 11 double bonds. Xanthophyll has two alcohol groups and kryptoxanthine one alcohol group. The structural formula for beta carotene was developed by Karrer (16) et al. The carotenoids are soluble in the ordinary fat solvents and insoluble in water, which accounts for their accumulation in material rich in lipoids. Carbon disulfide and chloroform are the best solvents. Schertz (17) gives the following table of solubility:

TABLE 1.—*Solubility at 25° C.*

SOLVENT	CAROTENE	XANTHOPHYLL
	mg./liter	*mg./liter*
Petroleum ether	626	9.5
Absolute ethyl alcohol	15.3	201.5
Absolute methyl alcohol	Nearly insoluble	134.9
Ethyl ether	1005	952.0

* Spectrophotometric terminology is given by the Committee on Spectrometry of the Optical Society of America (14).

The figures on solubility show the basis for the separation of carotene and xanthophyll by the Willstätter and Stoll (10) partition method, which specifies shaking a petroleum ether solution of these two pigments with aqueous methanol. By preferential solubility the carotene goes into the hydrocarbon layer and the xanthophyll in the aqueous methanol layer, which separation although not 100 per cent quantitative is sufficiently complete for most purposes. Such pigments as lycopene, carotenes, and xanthophyll esters go into the hydrocarbon layer, and the aqueous methanol layers contain the free xanthophyll, such as lutein, zeaxanthine, and bixin and crocetin.

The carotenoid pigments show different absorption spectra, which are more or less characteristic of the pigment. For alpha carotene in 80 per cent alcohol and 20 per cent ethyl ether, Smith (22) gives maximum absorption at wave length 4480 and 4760A°, and for beta carotene at 4530 and 4810A°. In the same solvent the values for xanthophyll read from Miller's (23) curves are 4470 and 4760A°.

Carotene and xanthophyll are fairly stable in petroleum ether and alcohol solutions, but carotene is the more stable. Schertz (17) found no appreciable change, by spectrophotometric measurements, for carotene in petroleum ether or in alcohol stored in an ice-box over a period of 150 days.

EXPERIMENTAL

Characterization of the Carotenoid Pigments of Flour by—

(a) *Application of the Willstätter and Stoll Petroleum Ether-Alcohol Partition Principle.*—As cited previously, Markley and Bailey reported that the carotene in four samples of wheat ranged from 12.8 to 34.8 per cent of the total pigment. The question naturally arises, What is the ratio for flour? Information on the range is necessary in concluding whether carotene has been added to flour or macaroni products.

Markley and Bailey's procedure for the separation of the flour pigments in 93 per cent naphtha and 7 per cent absolute alcohol was applied to several samples of flour. The emulsions formed were so disturbing and time-consuming that finally petroleum ether was substituted for the naphtha. Immediate separation with no emulsion resulted. Approximately 50 samples were analyzed by this procedure. The so-called carotene ranged from 5.0 per cent of the total color in durum flour to 54 per cent of the total color in the bread flours. The bleached flours showed a reduction in both the so-called carotene and xanthophyll fractions. While the so-called carotene results could be duplicated if the check analyses were made at the same time, it frequently happened that inconsistent results were obtained when the samples were re-analyzed at a later date. This suggested the possibility that saponification during the procedure might be a factor. Therefore the principle of a method of Guilbert (27)

was used for the determination of carotene. The method specifies the saponification and extraction of the pigments by heating on the steam bath with a potassium hydroxide saturated solution of ethyl alcohol. The results of analyses of 71 samples of flour and semolina gave a maximum carotene value of 0.20 p.p.m., and a large majority of the samples were around 0.10 p.p.m. These amounts, regarded as traces, led to further investigation of three samples of flour by the procedure of Markley and Bailey, but petroleum ether-alcohol solvent was used. Carotene values of 0.88, 0.90, and 0.90 p.p.m. were obtained. In order to test the affect of more complete saponification than that accomplished in the Markley and Bailey procedure on the amount of carotene found, the same solutions of the three flour samples were concentrated in a vacuum to about 10 cc. Two of the solutions were treated with 50 cc. of a saturated alcoholic potassium hydroxide solution on the steam bath and the separation was made according to the saponification method for determination of carotene. The results for so-called carotene were 0.12 and 0.19 p.p.m. The third solution was treated with 50 cc. of alcoholic potassium hydroxide and allowed to stand overnight at room temperature, and the separation was made as before, The result for the so-called carotene fraction was 0.10 p.p.m. Two samples of the same flour were extracted directly by the saponification method for determination of carotene. The results on the so-called carotene fraction were 0.13 and 0.14 p.p.m. This particular flour was chosen because the so-called carotene fraction represented 54 per cent of the total color by the Markley and Bailey procedure, one of the highest encountered. Other flours gave similar results.

Karrer and Ishikawa (28) (1930) have shown that xanthophyll is very commonly found as an ester of the fatty acids. Kuhn and Brockmann (29) (1932) have shown that the xanthophyll esters, helenien and physalien, would appear as carotene in the petroleum ether layer, and that the free xanthophyll would appear in the alcohol layer. It is concluded, therefore, that the high and inconsistent results obtained by the petroleum ether-alcohol partition method, similar to the one used by Markley and Bailey, are due to incomplete saponification of xanthophyll esters. One possible argument against this conclusion is that the small amount of so-called carotene was destroyed at the temperature of boiling alcoholic potassium hydroxide. Therefore, a solution containing 1.35 p.p.m. of carotene prepared from the crystals was added to the residue from this same flour, which had a so-called carotene content of 0.90 p.p.m. The added carotene obtained by the saponification method was 1.25 p.p.m., a 93 per cent recovery. These data, together with other experiments on larger amounts, show that carotene is not destroyed when heated to the boiling point of alcoholic potassium hydroxide for 30 minutes.

Judged on the basis of its behavior in the petroleum ether-methanol separation, practically all the carotenoid pigment in flour is xanthophyll,

and for the detection of added carotene to flour and macaroni products it may be assumed that this is true. Incidentally, the following findings on the results of bioassay tests on flour are of interest. Becker and von Hangai-Szabo (30) (1936) conclude that rats nourished only with flour show indications of a lack of vitamins A and D. In addition to the stoppage of growth, xerophthalmia frequently appeared, indicating feed poor in vitamin A. Since, according to the method of separation used here, the carotenoid pigment of flour is nearly all xanthophyll, there appears to be no possibility of the application of a method based on separation of the pigments for differentiation of bleached and unbleached flour. However, the method is useful in the detection of carotene added to macaroni products.

(b) *Application of Chromatographic Analysis.*—Because the proof that xanthophyll is the carotenoid pigment in flour is not definite, the following chromatographic procedure was followed. A 200 gram sample was treated by the saponification method, and the petroleum ether solution (moisture- and alcohol-free) of the pigment was poured onto a calcium carbonate adsorption tower. The adsorption was slight and quite unlike that of a solution of crystalline xanthophyll in petroleum ether. In a study of the use of different adsorbing agents for the separation of carotenoid pigments, Strain (31) (1934) found that a mixture of equal parts of magnesium oxide (special preparation) and "hyflo-supercell" is the most desirable for the separation of carotene and xanthophyll, or for separation of the carotene isomers. This mixture was accordingly used in continuation of this study, and the flour extract, prepared similarly to that used on the calcium carbonate tower, was poured onto the tower and slight suction was applied. The yellow band obtained at the top was somewhat scattered and was not like the band obtained from a solution of pure xanthophyll. Washing the column down with petroleum ether caused some separation, but it was not sufficiently definite to prove the presence of carotene. Although no satisfactory definite results were obtained by chromatographic analysis, the general behavior indicated that the pigment was xanthophyll. "Hydralo" was also tried, but no better results were obtained.

(c) *Absorption Spectrum of the Flour Extract.*—There seem to be four criteria for differentiation of the carotenoid pigments when in the crystalline form, namely: melting point, optical rotation, behavior of their solution toward adsorbing agents, and the characteristics of the spectral absorption curve. Unsuccessful attempts were made to crystallize the pigment from flour extract, and when the small amount of pigment present and the nature of flour are considered, it seems almost unreasonable to expect to obtain crystals. This eliminates the melting point and optical rotation. The behavor of the pigment on adsorbing agents was discussed under chromatographic analysis. This leaves for study the absorption

spectra of the flour extract. The adsorption was determined on a photoelectric spectrophotometer, at wave length intervals of 50A° from 4100–4900A°, on the xanthophyll fraction and on the carotene fraction of several flour extracts prepared by the saponification method used in this study. Presentation of the spectrophotometric data obtained will serve no useful purpose since the agreement with the values for absorption obtained by dissolving the crystalline pigment in solution is not close. However, the region of maximum absorption for the so-called xanthophyll fraction corresponds very closely with that for xanthophyll at 4450A° and fairly close at 4700A°. There is also good agreement in these regions with the xanthophyll fraction obtained from the treatment of fresh spinach by the same procedure. The so-called carotene fraction gave absorption maxima in the same position as did xanthophyll. This means that the trace of pigment left in the so-called carotene fraction is either unextracted xanthophyll or alpha carotene. Both fractions from the flour extract show an absorption maxima significantly different from that for beta carotene crystals in solution, and also from the carotene fraction obtained from the treatment of carrots and spinach by the same procedure. It is perhaps too much to expect that an extract prepared from flour should agree perfectly with the absorption curve obtained from xanthophyll crystals in solution. It is known that xanthophyll is present in spinach, and that carotene is present in spinach and carrots. However, there is the same lack of close agreement between the absorption curves of high purity crystalline material, and "carotene" extracted from spinach or carrots, or "xanthophyll" extracted from spinach. It would seem that there must be just enough interference to cause the variations observed with the flour extract or the spinach and carrots. Experiments that were conducted to prove that the method of treatment did not account for the variations found showed that it has no significant effect on the carotene and xanthophyll pigment.

The evidence from the absorption curves, from the behavior of the flour pigment upon application of the petroleum ether-alcohol partition principle, and from the chromatographic analysis strongly indicates that xanthophyll is the essential carotenoid pigment in flour. Certainly for purposes of detecting added carotene in flour and macaroni products it may be so considered.

SELECTION OF FILTER FOR NEUTRAL WEDGE PHOTOMETER

It is difficult to make a filter that will give strictly monochromatic light of a narrow wave length band and at the same time have a satisfactory degree of transmission. However, it is not essential that the absorption band be at 4358A°, as specified by Ferrari and Geddes in their spectrophotometric measurements of carotenoid pigments. This wave length has been used previously because it happens to be in the region of high ab-

sorption and is also an important component of the mercury arc, which of course is a powerful source of light. It is highly desirable to use some wave length in the region of maximum absorption, because (a) where the absorption is greatest one obtains most accurate measurements, particularly when the quantity of the absorbing substance is small; and (b) it is this region that contains the wave length peculiarly characteristic of the molecule.

Miller (20, 23) (1934) published the quantitative absorption spectra curves of the common carotenoids: alpha carotene, beta carotene, lyco-

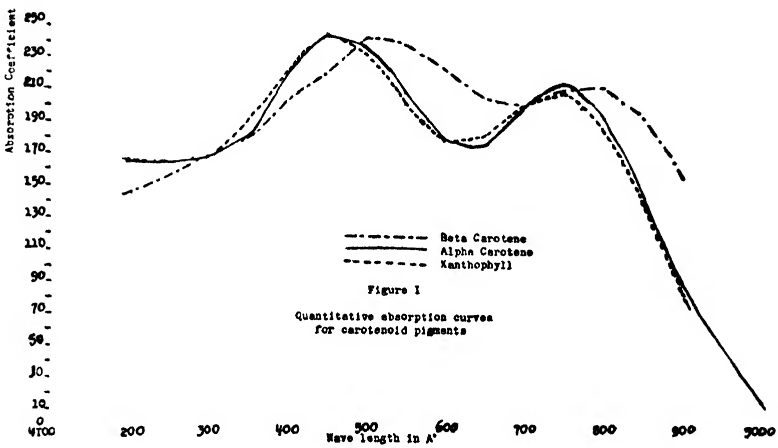


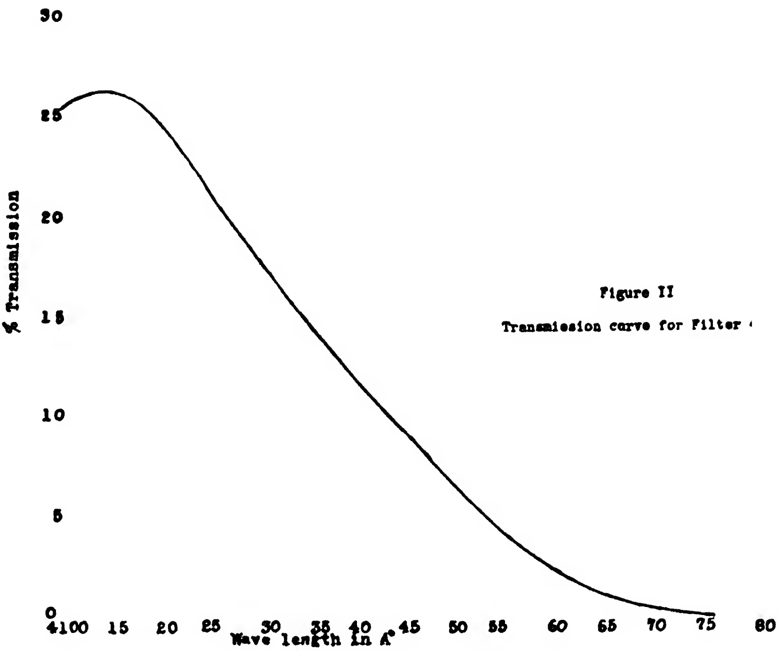
Figure I

Quantitative absorption curves for carotenoid pigments

pene, and leaf xanthophyll. In the present study on alpha and beta carotene and xanthophyll, in an 80 per cent alcohol and 20 per cent ether mixture, using a photoelectric spectrophotometer, the writer obtained a set of curves (Fig. 1) in close agreement with those of Miller. However, at 4450A° Miller's curves show the absorption coefficients to be equal for alpha carotene, beta carotene, and xanthophyll, while in Fig. 1 they are only approximately so. On the basis of Miller's results it should make no difference from the standpoint of the calculation of concentration from transmittancy measurements, which pigment is measured, alpha carotene, beta carotene, or xanthophyll at wave length 4450A°. Therefore, since it is not positively known which pigment constitutes the coloring matter of flour, approximately 4450A° was chosen for this work.

A No. 511 Corning glass filter, 5 mm. in thickness, was found to have an optical centroid or wave length center of gravity of the transmitted light of 4444A°, which is very close to 4450A°. It will be referred to hereafter as filter No. 44. This optical centroid was determined according to Foote (32). The transmission curve for this filter is given in Fig. 2.

According to the absorption curves for the carotenoid pigments, equal concentrations at 4450A° should have the same absorption coefficients. If it is assumed that the optical centroid of the No. 44 filter is correct as



measured, that the pigments are pure, and that the error in the concentrations prepared is negligible, the three solutions of alpha and beta carotene and xanthophyll of equal concentrations should read the same on the neutral wedge photometer. Table 2 shows the results of reading equal concentrations of the three pigments in a mixture of 80 per cent absolute alcohol and 20 per cent anhydrous ethyl ether.

TABLE 2.—*Photometer scale readings for equal concentrations of alpha and beta carotene and xanthophyll (No. 44 filter)*

	Scale Reading on Photometer		
Concentration *mg./liter*	*Alpha Carotene*	*Beta Carotene*	*Xanthophyll*
0.30	3.61	3.64	3.43
0.40	4.99	4.95	4.74
0.50	6.02	6.05	5.71

The values for alpha and beta carotene agree well, but the xanthophyll values are low, which is in agreement with the absorption data for xanthophyll, indicating a slight impurity in this pigment.

METHOD FOR THE DETERMINATION OF ADDED CAROTENE IN FLOUR AND MACARONI PRODUCTS

STANDARDIZATION OF NEUTRAL WEDGE PHOTOMETER FOR MEASURING CAROTENE CONCENTRATION

Dissolve 100 mg. of a natural mixture of alpha and beta carotene in 5–6 cc. of CS_2, add 35–40 cc. of absolute ethyl alcohol, cool in ice-box for about 1 hour to

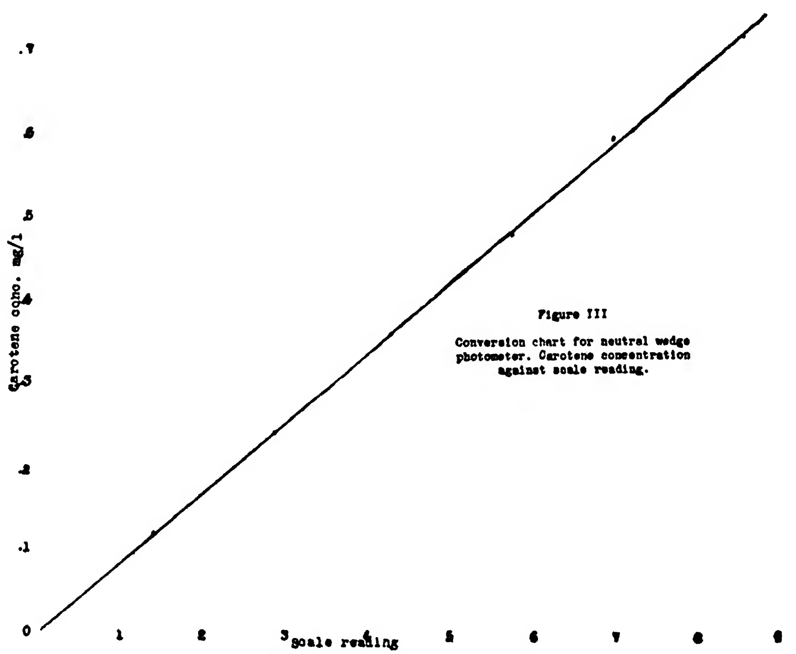
insure maximum crystallization, and filter on a *hard* filter paper. Dissolve the carotene crystals in 5–6 cc. of CS_2, add 40 cc. of petroleum ether, cool in ice-box as before, filter on a *hard* filter paper, and dry crystals in a vacuum desiccator for 1 hour.

Weigh accurately 20 mg. of purified crystals and wash with 20 cc. of anhydrous ethyl ether into a 1000 cc. graduated glass-stoppered flask. Continue to wash with petroleum ether, and make to volume by adding petroleum ether as soon as the carotene completely dissolves. Designate this as stock solution.

Make up eight concentrations by adding the following amounts of this stock solution to a 250 cc. graduated flask: 1.25, 2.50, 3.75, 5.00, 6.25, 7.5, 8.75, and 10.00 cc. Make to volume with petroleum ether. These dilutions represent concentrations of 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80 mg. per liter. Read these solutions in a 4-inch cell, using filter No. 44 in the photometer eyepiece. (The cell, filled with the solvent, should read zero on the photometer scale.) Make 10 readings on each solution and plot average reading against concentration. The line of best fit for the data is obtained by the method of least squares.* In application of this method let x represent the scale reading and y the concentration in mg. per liter. Substitute in the following expressions for the value of a and b to give the equation $y=a+bx$.

$$b=\frac{\Sigma xy-n\ M_xM_y}{\Sigma(x)^2-n\ (M_x)^2}\ \text{and}\ a=M_y-b\ M_x$$

Do the standardization the same day the stock solution is prepared, and standardize each neutral wedge photometer.



* This gives a straight line as in Fig. 3, which indicates that Beer's law is obeyed over this range of concentrations. Scherts (5) has also shown that Beer's law holds up to 3.0 mg./l.

PREPARATION OF SAMPLE

Grind macaroni and noodles to as near flour fineness as possible in the ordinary coffee type mill. No difficulty occurs with products containing egg, but plain macaroni products require several grindings. Take care not to set the mill too tight, as the heat generated may be sufficient to damage the pigments.

REAGENTS

Petroleum ether.—B.P. 30–60.

Ethyl ether.

Alcoholic potassium hydroxide solution.—Saturated, 10 grams of KOH per 100 cc. of 95% alcohol.

Methyl alcohol.—92% 8 cc. of H_2O +92 cc. of absolute methyl alcohol.

Anhydrous sodium sulfate.

APPARATUS

125 cc. separatory funnels.

250 cc. separatory funnels.

250 cc. distillation flask.

Mechanical shaker (desirable).

Neutral wedge photometer and absorption cells.—2 and 4 inch.

30 cm. condenser.

250 cc. suction flask.

Büchner type fritted glass filter (11G3).

PROCEDURE

Weigh 20 grams of flour, semolina, or macaroni, or 10 grams of egg noodles, or 2 grams of egg yolk, into a 125 cc. Erlenmeyer flask, add 50 cc. of the saturated alcoholic KOH, and heat on a steam bath for 30 minutes with the flask fitted to a reflux condenser. Rotate the flask occasionally during this time, but be as careful as possible to keep the sample from collecting on the sides of the flash. Remove the flask, and cool to room temperature. Filter the solution through a Büchner type fritted glass filter (11G3), using suction, transferring all material with a little 95% alcohol from a wash bottle. Turn off suction, rinse out flask with 25 cc. of ethyl ether, and pour the rinsings into the glass filter, stirring the material with a rod to allow the ether to come in contact with all portions. Filter off and repeat this procedure twice more. Transfer the filtrate to a 250 cc. glass-stoppered separatory funnel, rinsing with about 25 cc. of ethyl ether. Disregard any resinous material. Add 175 cc. of cold tap water, carefully invert, and rotate several times. When the aqueous-alcohol and ether layers have separated, remove the lower aqueous-alcohol layer and extract this layer again with 25 cc. of ethyl ether. Discard the lower layer. Add the ether to the original ether solution. Wash the ether by pouring 50 cc. of tap H_2O through it. After separation of the layers, withdraw the H_2O layer and discard it. Add to the ethyl ether solution 50 cc. of petroleum ether and wash 5 times with 50 cc. of tap H_2O, shaking gently by inverting and rotating the funnel. Discard all H_2O layers. Slight emulsions may occur but can be disregarded.

Transfer the ether-petroleum ether mixture to a 250 cc. distillation flask, and place the flask in a beaker of H_2O at 45–50° C. Stopper the flask, connect the side arm with a vacuum, and concentrate to about 5 cc. to remove the ethyl ether. Filter through a paper two-thirds filled with anhydrous Na_2SO_4, into a 100 cc. volumetric flask. Make to volume with petroleum ether that has been passed portion-wise through the filter and Na_2SO_4. (If a total color determination is desired, fill the 4-inch absorption cell with a pipet, since none of the solution must be lost during the filling, and read in the neutral wedge photometer, using filter No. 44. Return the solution in the cell to the flask.) Transfer the solution to a 125 cc.

separatory funnel, rinsing with a little petroleum ether. Add 15 cc. of 92 per cent methyl alcohol, shake on a mechanical shaker for 10 minutes, or 2–3 minutes by hand, remove, and allow the separatory funnel to stand in an upright position a minute until the alcohol and ether layers separate. Decant the lower layer containing xanthophyll and repeat the extraction 5 more times, or until the aqueous methyl alcohol layer is colorless. (It is necessary to examine the final methyl alcohol layer in a test tube over a white background to be sure it is colorless. (Six extractions are usually sufficient on flour and macaroni, but noodles with high egg content may require 10 extractions.)

Wash with 25 cc. of H_2O, inverting the separatory funnel several times, decant, and discard the H_2O layer. Repeat twice more. After the last washing pour the petroleum ether layer into the 250 cc. distillation flask. Place the flask in a beaker of H_2O at 45–50° C., stopper the flask, and concentrate the petroleum ether by vacuum as before. Concentrate to 10–15 cc. and filter through a dry filter paper two-thirds filled with anhydrous Na_2SO_4 into a 25 or 50 cc. volumetric flask, depending on concentration of carotene. Wash the filter paper with petroleum ether in making to volume. Mix contents and read the concentration in a 4-inch absorption cell in a neutral wedge photometer, using filter No. 44. From the standardization curve, read off the concentration of carotene. Divide by 4 or 2, depending on volume adjustment, to obtain concentration of carotene in 100 cc. Multiply by 5 to convert to p.p.m. in the sample if a 20 gram sample was used, and by 10 for a 10 gram sample, etc.

The total color value calculated as carotene may be obtained, when desired, from the same standardization curve, since carotene and xanthophyll show nearly identical absorption with the No. 44 filter. It represents approximately the amount of xanthophyll and carotene. The dilution for total color is already 100 cc. Therefore to convert to p.p.m. in the sample multiply by 5 if a 20 gram sample was used, and by 10 for a 10 gram sample, etc.

PRECISION OF THE METHOD

The results of replicate analyses by this method of samples of macaroni and noodles containing added carotene are given in Table 3. These samples were analyzed at different intervals and may be considered as representative of the precision of the method. Total color is calculated as carotene.

TABLE 3.—*Results on macaroni and noodles with added carotene*

(Expressed in p.p.m.)

				Macaroni				
Total Color	2.48,	2.65,	2.35,	2.44				
Carotene	1.00,	1.05,	0.88,	0.92				
Total Color	3.20,	3.20,	3.45,	3.35,	3.20,	—	—	—
Carotene	1.50,	1.56,	1.53,	1.56,	1.58,	1.64,	1.52,	1.80
Total Color	9.10,	9.40,	9.20,	8.60,	9.0			
Carotene	4.45,	4.30,	4.60,	4.50,	4.45,	4.40		
				Noodles				
Total Color	15.8	—	—	—	15.3,	16.4		
Carotene	4.40,	4.26,	4.50,	4.50,	4.50,	4.30		

The average deviation of a single result is the sum of the deviations divided by the number of results, the deviation being the difference between the average of all results and a single result. The average deviation for macaroni with the intermediate amount of added carotene (1.58) is 0.07 p.p.m. The arithmetical mean derived from n measurements of equal reliability is $\sqrt{n}$ times as reliable as any single result. The reliability of the mean of two results is, therefore, $.07/\sqrt{2}$, or 0.05 p.p.m. The same calculation applied to the macaroni with a larger amount of added carotene (4.43) gives an average deviation for a single result of 0.07 p.p.m. The average deviation of the mean of two results is thus 0.05 p.p.m. The precision of the method for determination of added carotene may, therefore, be considered good. In general, the reliability of a single determination may be considered to be very close to plus or minus 0.10 p.p.m. The values for total color are not so precise, but they are not of primary interest in this study.

ACCURACY OF THE METHOD

A carotene solution prepared from crystalline carotene containing 0.72 mg. per liter was added to 50 cc. of alcoholic potassium hydroxide. The recovery was 0.695 mg. per liter, or 96.5 per cent. The same carotene solution plus an amount of crystalline xanthophyll corresponding to that in 20 grams of flour was treated likewise, and the recovery was 0.725 mg. per liter, or 100.6 per cent. These recoveries are considered very good, and there was no interference. The accuracy of the method when applied to flour containing known amounts of added carotene was next tested. A sample of flour previously analyzed by this method and found to have a small carotene blank was used. Two different concentrations of carotene solutions were added to 20 grams of this flour and recovered by the method. The amounts of carotene added were 0.72 and 0.30 mg. per liter, and the amounts recovered as carotene, after deduction of the small blank, were 0.68 and 0.28 mg. per liter, respectively, or 94.5 and 93.4 per cent. Further experiments to test the accuracy of the method consisted in extracting this same flour with 93 per cent petroleum ether and 7 per cent alcohol overnight, filtering off the flour residue, washing well with petroleum ether, and drying; 20 grams of this flour residue was added to a carotene solution of 0.54 and 0.27 mg. per liter. Then to each sample was added a xanthophyll solution (prepared from the crystals) corresponding to the amount of xanthophyll in the flour originally. The recoveries of carotene by the regular method were 0.50 and 0.25 mg. per liter, or 92.6 and 92.6 per cent, respectively. These results indicate that a recovery of at least 92 per cent of the added carotene can be obtained when there is present at least 3.60 p.p.m. of this pigment on the flour basis (0.72 mg./liter $\times 5$).

The total color calculated as carotene may be used in estimating the

TABLE 4.—*Total color and carotene in flour, farina, and semolina*
(Results expressed in p.p.m. calculated as carotene)

NO.	SUBSTANCE	TOTAL COLOR	SO-CALLED CAROTENE
1	Flour	—	0.10
2	"	—	0.08
3	"	—	0.07
4	"	—	0.07
5	"	—	0.08
6	"	—	0.10
7	"	—	0.15
8	"	—	0.08
9	"	3.3	0.13
10	"	3.4	0.12
11	"	3.65	0.12
12	"	—	0.10
13	"	—	0.10
14	"	—	0.13
15	"	—	0.10
16	"	—	0.10
17	"	—	0.15
18	"	—	0.12
19	"	—	0.12
20	"	—	0.08
21	"	—	0.13
22	"	—	0.09
23	"	—	0.10
24	"	—	0.10
25	"	—	0.15
26	"	3.45	0.20
27	"	3.20	0.18
28	"	2.60	0.10
29	"	2.65	0.15
30	"	3.20	0.18
31	"	2.90	0.16
32	"	2.15	0.12
33	"	2.50	0.12
34	"	2.30	0.13
35	"	2.13	0.12
36	"	2.45	0.12
37	"	1.75	0.05
38	"	2.05	0.08
39	"	1.75	0.09
40	"	2.23	0.12
41	"	3.0	0.11
42	"	2.7	0.12
43	"	2.8	0.13
44	"	2.5	0.08
45	"	3.1	0.10
46	Farina	2.3	0.08
47	"	2.45	0.13

TABLE 4 (*Continued*)

NO.	SUBSTANCE	TOTAL COLOR	SO-CALLED CAROTENE
48	"	2.15	0.05
49	"	2.60	0.08
50	"	—	0.10
51	Semolina	—	0.10
52	"	—	0.08
53	"	—	0.10
54	"	3.45	0.08
55	"	2.75	0.10
56	"	2.80	0.14
57	"	2.75	0.10
58	"	2.90	0.11
59	"	3.12	0.08
60	"	2.60	0.08
61	"	2.50	0.05
62	"	2.64	0.10
63	"	2.75	0.14
64	"	2.40	0.12
65	"	2.20	0.10
66	"	2.94	0.10
67	"	3.20	0.06
68	"	3.42	0.08
69	"	5.20	0.20
70	"	3.90	0.20
71	"	4.95	0.20

amount of xanthophyll, which is taken to be the difference between the total color and carotene.

RESULTS OF DETERMINATION OF SO-CALLED CAROTENE IN UNBLEACHED FLOURS

In order to detect the addition of carotene to macaroni and noodles it is necessary to know the natural range of the so-called carotene in flours. The results in Table 4 were obtained on 71 samples of various flours representing commercial blends going to the macaroni industry and individual varieties experimentally milled by D. A. Coleman, U. S. Department of Agriculture. The results indicate that the fraction measured as carotene by this method will not exceed 0.2 p.p.m.

Eggs, a normal ingredient of noodles, contain a certain amount of pigment that remains in the petroleum ether layer and is therefore calculated as carotene. Gillam and Heilbron (33) report this pigment to be largely kryptoxanthine, with very little carotene. One of their analyses gives 20 p.p.m. total pigment, 1.9 p.p.m. kryptoxanthine, and 0.15 p.p.m. carotene. The color of individual egg yolk varies from a pale yellow to a deep reddish orange. The variation in 17 authentic commercial frozen yolks and dried yolks is shown by the figures for total color in Table 5.

The minimum amount of egg solids specified in the standard for noodles is 5.5 per cent on the dry basis, or 0.55 gram of egg solids in 10 grams of the noodles used in this procedure for determination of color. On the basis of the carotene figure found on the egg sample of highest color, Table 5, there might be expected a so-called carotene figure of 0.55 p.p.m. on an egg noodle, chargeable to the egg. Then there is a slight amount of carotene chargeable to the flour itself. This carotene blank will vary, depending on the amount of egg solids, the color of the egg, and the color of the flour.

TABLE 5.—*Results for total color (calculated as carotene) and carotene on authentic samples of eggs from various parts of the country*

NO.	SUBSTANCE	TOTAL COLOR (MOISTURE-FREE BASIS)	"CAROTENE" (MOISTURE-FREE BASIS)
		p.p.m.	*p.p.m.*
1	Dried egg yolk	78.0	6.2
2	" " "	47.5	3.7
3	" " "	41.0	4.1
4	" " "	57.7	4.6
5	Frozen egg yolk	81.5	6.0
6	" " "	154.0	10.3
7	" " "	54.4	3.8
8	" " "	66.6	4.2
9	" " "	62.3	5.0
10	" " "	99.2	6.4
11	" " "	91.0	6.0
12	" " "	101.0	6.5
13	" " "	98.0	5.5
14	" " "	48.4	3.1
15	" " "	45.0	2.4
16	" " "	51.0	2.6
17	" " "	55.0	3.0

The last four samples of frozen eggs (Table 5) are Pacific Coast eggs. The values for both total color and carotene fraction are considerably below those on eggs from other sections of the country. However, on these limited data no generalizations can be made.

The analyses of some representative commercial samples of macaroni (Table 6) give an idea of the usual range of total color and carotene fractions. These results may be lower than those obtained on the original raw material, due largely to destruction of the pigment during the drying process.

The results of Table 6 may be of value from the standpoint of establishing a maximum value for total color in macaroni, but the minimum value may be very low indeed. The carotene fractions are of the same order of magnitude as those obtained on flour.

TABLE 6.—*Total color and carotene in commercial macaroni*

SAMPLE NO.	TOTAL COLOR	CAROTENE FRACTION
	p.p.m.	*p.p.m.*
1	2.1	0.15
2	2.5	0.16
3	2.1	0.16
4	0.6	no color
5	3.0	0.16
6	1.6	0.15
7	1.6	0.05
8	2.0	0.10
9	1.8	0 05
10	1.7	0.08
11	1.2	0.08
12	0.5	0.05
13	1.0	0.06
14	1.4	0.08
15	2.0	0.09
16	1.8	—
17	2.1	0.16
18	2.9	0.18
19	3.0	0.18

TABLE 7.—*Total color and carotene in commercial noodles*

NO.	TOTAL COLOR	CAROTENE FRACTION
	p.p.m.	*p.p.m.*
1	12.10	0.80
2	8.2	0.60
3	11.6	0.70
4	9.8	0.80
5	7.6	0.50
6	6.6	0.50
7	7.2	0.60
8	10.6	0.60
9	11.0	0.60
10	8.0	0.50
11	8.8	0.60
12	11.4	0.50
13	11.2	0.60
14	10.7	0.50
15	8.1	0.60
16	11.0	0.70
17	8.6	0.50
18	5.4	0.30
19	5.6	0.40
20	10.4	0.70
21	11.6	0.70
22	6.2	0.50
23	12.4	0.80

The results on representative commercial noodles, Table 7, likewise show quite wide variation in total color, with the carotene fraction less variable. In preparation there is less destruction of the pigment of noodles than of macaroni, as shown by experiment, and the difference, therefore, is largely due to variation in color of raw material and in amount of egg solids.

The analyses of 71 samples of raw materials used in macaroni manufacture and 19 samples of macaroni show a maximum value for the caro-

TABLE 8.—*Total color and carotene in authentic macaroni products*

	TOTAL COLOR	CAROTENE FRACTION
	p.p.m.	*p.p.m.*
Flour used in products (1–11 inclusive)	4.45	0.18
1 Macaroni	4.03	0.20
2 Macaroni with added carotene	9.4	4.30
3 Macaroni with added carotene	8.4	4.20
4 Noodles with 3% frozen egg yolk solids	7.7	0.25
5 Noodles with 5.5% frozen egg yolk solids	10.9	0.35
6 Noodles with 8.0% frozen egg yolk solids	16.2	0.53
7 Noodles with 11.0% frozen egg yolk solids	18.8	0.60
8 Noodles with 5.5% whole egg solids	10.2	0.45
9 Noodles with 11.0% whole egg solids	14.8	0.55
10 Noodles with 5.5% egg yolk solids plus carotene	16.4	4.30
11 Noodles with 5.5% dried egg yolk solids	7.7	0.43
Flour used in products (12–14 inclusive)	2.23	0.12
12 Macaroni	1.15	0.11
13 Macaroni with added carotene	2.35	0.88
14 Macaroni with added carotene	3.20	1.50

tene fraction of 0.20 p.p.m. Generally it was much lower. As an authentic sample a noodle (No. 7, Table 8) with 11 per cent egg solids was prepared from a highly colored egg yolk. This sample gave a carotene fraction of 0.60 p.p.m., which may be considered close to the higher value expected on a commercial noodle. However, Table 7 contains seven values somewhat higher than 0.60, the highest being 0.80 p.p.m. These samples are ordinary commercial noodles offered to the retail trade. Table 8 gives total color and carotene content of authentic factory-prepared samples of macaroni and noodles. These results indicate the range of carotene value in products having added carotene, with or without egg.

INTERFERENCE

Since other colors may be added to noodles, it was necessary to determine the influence of these colors on the determination of added carotene.

The usual colors added are annatto, turmeric, saffron, tartrazine, naphthol yellow S, yellow AB, and yellow OB. In addition, orange I and sunset yellow FCF were tested. Naphthol yellow S, turmeric, saffron, orange I, sunset yellow FCF, and tartrazine were added to macaroni and analyzed by the method. Since all color was removed in the alcohol-water layer, the total color was unchanged. Annatto treated likewise was largely removed in the alcohol-water layer, any remaining being removed by the methanol, and thus it caused no interference in the carotene determination. Yellow AB and OB are both oil-soluble dyes that remain in the petroleum ether layer and are therefore measured as total color. They do, however, act similarly to xanthophyll, being removed in the aqueous methyl alcohol solution. Their presence, therefore, does not interfere with the measurement of the carotene fraction.

TABLE 9.—*Gasoline color values obtained by comparison with a standard of arbitrarily assigned value of 1.0*

	1	2	3	4
Analyst 1	.94	.43	1.49	1.91
2	1.05	.45	1.53	1.95
3	1.31	unable to compare	2.06	2.22
4	1.31	.52	2.49	2.49
	Photometer values in p.p.m. calculated as carotene			
Analyst 1	1.32	.49	2.37	3.65
2	1.32	.44	2.54	3.89
3	1.34	.46	2.42	3.82
4	1.32	.49	2.48	3.82

FURTHER APPLICATION OF THE NEUTRAL WEDGE PHOTOMETER

By affording a convenient and accurate measurement of the total color of flour extract, the neutral wedge photometer is an aid in the differentiation of bleached and unbleached flour. Winton's gasoline color value method has given variable results. A comparison of results by the Winton gasoline color values and those obtained on the photometer is given in Table 9. The same four solutions were compared in the colorimeter by the Winton procedure and then read in the neutral wedge photometer by four different analysts. There is considerably better agreement among analysts with the latter procedure.

This photometer can be used to measure the carotene extracted from various food products and feeds, in connection with studies on its relation to vitamin A. In like manner it can be used to measure xanthophyll and chlorophyll.

SUMMARY

Evidence has been presented to show the need of a relatively cheap instrument for the accurate measurement of the added carotene in flour

and macaroni products. The application of the neutral wedge photometer to such measurement has been described. It involved a study of the pigment naturally occurring in flour, of the selection of a filter for the photometer, and of the standardization of the photometer against pure carotene solution. A method for the extraction of added carotene from flour and macaroni products is presented. It shows high precision and good accuracy. The application of the method to the detection of added carotene requires a comprehensive study of raw materials and finished products. Results by this method on total color and carotene in flour, farina, semolina, macaroni, egg yolks, and noodles are shown. From these results it is possible to select a value above which it can be concluded that carotene has been added.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Dr. L. B. Broughton and Dr. N. L. Drake for suggestions and constructive criticism. Thanks are also due to Mr. P. A. Clifford for assistance in construction and advice on the neutral wedge photometer and to Dr. B. A. Brice for advice on various physical problems.

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MICROSCOPIC IDENTIFICATION OF SULFANILAMIDE

By MORRIS L. YAKOWITZ (U. S. Food and Drug Administration, San Francisco, Calif.)

Sulfanilamide reacts with aromatic aldehydes at room temperature. The reaction products formed with benzaldehyde and cinnamon oil may be used for identifying sulfanilamide. The following tests are presented:

With Benzaldehyde

Place a drop of benzaldehyde on a microscope slide and stir in about 1 mg. of powdered sulfanilamide. Stir the mixture occasionally for about a minute, or until the reaction product appears, then examine under the microscope at 100×. The reaction product will appear as small smooth plates having the outline of a parallelogram. The plates will grow to a good size within several minutes. The parallelograms have the acute angle = 68° with the obtuse angle = 112°.

With Cinnamon Oil

Place a drop of cinnamon oil on a microscope slide and stir in about 1 mg. of powdered sulfanilamide. Examine under the microscope at about 100×, using strictly axial illumination. After several minutes the reaction product will appear in the form of plate-like crystals. As the plates rotate they disappear from view when the flat faces are presented to the observer. As the plates change this position, they gradually come back into view until maximum visibility or "relief" is obtained, when the crystals present an edge view to the observer.

Some of the plates acquire roughly the shape of an elongated hexagon, but most of them do not have any distinctive outline. However, the behavior described is common to all the plates.

BOOK REVIEWS

Handbook of the Science of Eggs (Handbuch der Eierkunde). By J. GROSSFELD. 375 pp., 45 illustrations. Julius Springer, Berlin, 1938. Price unbound 27 RM, bound 28.50 RM.

One familiar with the science of eggs readily agrees with the author that this compilation finds no counterpart in the world's literature and should find full use by the investigator, producer, and user of eggs.

The book is replete with references covering many years and including contributions from all over the world. Glancing through the citations, the writer noted dates as far back as 1810, but he was most impressed by the number of contributions of

recent origin, including many in the year 1937, and it was with some pride that literally scores of references to the reports of American authors were observed.

The six large chapters of the book are devoted to the broad subjects of physiology and morphology, composition, preservation, digestibility and nutritive value, German statistics and regulations, and examination of eggs and egg products. Omissions of references which might be regarded as pertinent to the subjects treated are so few as not to merit mention of specific instances. However, very little information is included on the bacteriology of eggs and there is no discussion of egg grading, a field perhaps more fully developed in this country than in Europe. The wish of the author, that the book may be of help to every colleague and at the same time give a picture of the present status of research on the theoretical and practical aspects of the subjects, is fulfilled.—HENRY A. LEPPER.

TUESDAY—MORNING SESSION

An informal abstract of the reports under plants was given by E. J. Miller. The former Referee on Plants, O. B. Winter, East Lansing, Mich., died October 1, 1937. An obituary of Mr. Winter appeared in the February, 1938, number of *This Journal.*

No report on less common metals in plants was given by the associate referee.

REPORT ON TOTAL CHLORINE

By HERBERT L. WILKINS (Bureau of Plant Industry, Division of Forage Crops and Diseases, National Agricultural Research Center, Beltsville, Md.), *Associate Referee*

No collaborative studies were made on the tentative method, *This Journal,* **19,** 72–4, 366–71 (1936); *Ibid.,* **20,** 335–7 (1937); *Methods of Analysis A.O.A.C.,* 1935, 131, as presented previously by the Associate Referee. Several variations of benzene solutions of iodine were prepared, and while some were satisfactory others were not. Among the more satisfactory solutions were those that had been washed repeatedly with either water or boiled and cooled dilute sulfuric acid. One of the chief difficulties with the benzene solutions is that particles of undissolved iodine remain in the titration flask in spite of vigorous shaking. They appear to react with the starch, forming the blue color. Different lots of iodine also gave solutions of varying quality, some of which instantly produced a color in the test solution. Toluene and n-butyl alcohol were also tried as solvents.

When the boiled and cooled dilute sulfuric acid was used to prepare an iodine solution it was decanted from the crystals and a portion was tested by titration with standard potassium iodide and silver nitrate solutions. The indicated amount of the silver nitrate was added to the remaining solution and tested again. To the resulting solution, which was substantially in equilibrium, a considerable excess of silver nitrate was added. The test of course showed this excess, but it was nearly absent after the solution had stood a few hours and was found to be in excellent equilibrium the next morning. Another similar solution was made, but it was treated with twice the indicated amount of silver nitrate and left standing over the crystals of iodine. The test showed it to be in satisfactory equilibrium both immediately and after standing overnight.

That all soluble starches are not equally suitable was shown when a new supply from a different manufacturer was found to froth considerably and to give a very opalescent solution after boiling 10 minutes. Two hours of boiling improved the solution, but it did not make it so satisfactory as

the solutions formerly prepared with 5 minutes' boiling. With this starch it was apparently more difficult to discharge the starch-iodide color with silver nitrate because an unusual excess was required. Starch from still another manufacturer was found to be quite satisfactory.

When titrations were made with standard solutions one-tenth as strong as those given in the tentative method (0.00282 *N*), in one-tenth of the total volume of liquid, and with a like reduction in the amounts of starch and iodine solutions, the end points were just as good as when they were obtained in the regular way. It would seem possible, therefore, to estimate small amounts of chlorine with a precision of ± 0.002 mg. and with an accuracy of about ± 0.005 mg. if the material and other conditions would allow the titration to be made in this small volume.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative studies be made on the modifications of the tentative method suggested since its publication.

(2) That a report of any difficulties encountered in the use of the method be made to the Associate Referee as soon as possible.

No report on carbohydrates in plants was given by the associate referee.

No report on inulin in plants was given by the associate referee.

No report on forms of nitrogen in plants was given by the associate referee.

REPORT ON HYDROCYANIC ACID IN PLANTS*

By ROBERT A. GREENE (Arizona State Laboratory, Tucson, Ariz.), *Associate Referee*

After consideration of the difficulties involved in a collaborative study of this nature, and after correspondence with the referee, the Associate Referee decided to present a method of analysis for consideration at this time, and to undertake some form of collaborative work at a later date.

The methods that are presented have been used extensively in the past few years in connection with a study of the occurrence of cyanogenetic glucosides in the range plants of Arizona. In spite of the difficulties involved in securing and transporting samples, the methods have given satisfactory results.

[1] For report of subcommittee A and action by the Association, see *This Journal*, 21, 63 (1938).
* Presented by L. D. Haigh.

QUALITATIVE TESTS

The first method presented was originally suggested by Guignard[1] and the technic is given by Morrow.[2] The test is conducted in the following manner:

Prepare sodium picrate paper by dipping strips of filter paper into 1% aqueous picric acid solution and allowing to dry, and then dipping in a 10% solution of $NaCO_3$ and drying. Preserve the papers in a tightly stoppered bottle.

Place a portion of the plant to be tested in a test tube. (Better results are obtained if the material is finely chopped immediately before being placed in the tube.) Add a few drops of $CHCl_3$ and insert a strip of moistened sodium picrate paper, taking care that it does not come in contact with the material being tested. Close the tube with a tightly fitting cork and allow to stand. The sodium picrate paper turns orange and then brick red if cyanogenetic glucosides are present.

The preceding test is very satisfactory for use in the field, but the test proposed by Fox[3] is more sensitive and specific and has given excellent results in this laboratory. It is conducted as follows:

Allow the sample to autolyze with four or five times its weight of distilled water, and then aspirate the HCN formed into one drop of 5% KI solution. Add one drop of 0.001 *M* $AgNO_3$ and then 1 ml. of 5% NaOH, which are contained in a small test tube. The faint bluish cloud of AgI disappears and the solution becomes crystal clear if the plant contains hydrocyanic acid.

Fox found that with a more dilute silver nitrate solution (0.00025 *M*) 1 part of hydrocyanic acid in 2,000,000 can be detected.

QUANTITATIVE TESTS

Take the usual precautions to insure that the sample is representative, and if the plants are to be transported for any distance, take care to avoid brusing or crushing. (It is preferable, when possible, to secure the samples in the field.) Chop the material fine, weigh (portable balances are available), and place in a flask with four to five times its weight of water. Stopper the flask tightly and allow the contents to autolyze. To prevent any loss of HCN during a transfer, it is advisable to collect the sample in a flask of the same size as will be used in the distillation process.

If the sample can be brought to the laboratory, grind or finely chop it as rapidly as possible. (The size of sample will depend upon the HCN content of the material. A sample of 100 grams usually gives satisfactory results.)

After the sample has autolyzed for 4–8 hours, determine the HCN by steam distillation, according to the methods for the determination of hydrocyanic acid formed by the hydrolysis of glucosides in grain and stock feeds, *Methods of Analysis, A.O.A.C.*, 1935, 347.

In order to continue study of this subject it is recommended[4] that the methods suggested here be adopted as tentative.

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[1] *Bull. sci. pharmacol.*, 13, 415 (1906).
[2] Biochemical Laboratory Methods, John Wiley and Sons (1927).
[3] *Science*, 79, 237 (1934).
[4] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 63 (1938).

REPORT ON SODIUM AND POTASSIUM

By R. T. MILNER (U. S. Regional Soybean Industrial Products Laboratory, Urbana, Ill.), *Associate Referee*

During the past year no collaborative work was done on sodium and potassium. The present rapid method for potassium only, *Methods of Analysis, A.O.A.C.*, 1935, 126, gave excellent results on soybean seed. It was found safer, however, to use sugar, as specified in the procedure for fertilizers, *Ibid.*, 30, **44(a)**, to prevent spattering in the process of evaporation. Efforts to use the Hicks method[1] were unsuccessful as low results were always obtained. Further work will be done on this method. In this laboratory the platinum is recovered successfully by the use of magnesium ribbon.

A personal communication from C. H. Robinson, Agricultural Chemist, Dominion of Canada, gave data showing the necessity of saturating the uranyl acetate reagent with sodium magnesium uranyl acetate before use. Without this precaution results for sodium are a little high. This information is supported by an examination of the original data, *This Journal*, **17**, 275 (1934), and by experiments in this laboratory. Further study will be made on this determination.

The present official method for sodium and potassium in plants, *Methods of Analysis A.O.A.C.*, 1935, 125, **14**, is laborious and apparently rarely used. If the method specifying sulfuric acid in ashing can be replaced by another method, such as that using nitric and perchloric acids, the tentative perchloric acid method for sodium and potassium, *Ibid.*, 126, **16**, can be considerably improved and paragraph **14** can be deleted.

RECOMMENDATIONS[2]

It is recommended—

(1) That work be continued on the Hicks method for the determination of potassium only.

(2) That in the tentative method for the determination of sodium only, page 126, par. **18**, the section reading "allow to cool to 20°" be changed to read, "allow to cool to about 30°."

(3) That the present tentative perchloric acid method for the determination of sodium and potassium, page 126, **16**, be studied with the view to shortening and improving the procedure for preparation of solution.

REPORT ON LIGNIN

By MAX PHILLIPS (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.), *Referee*

In view of the fact that it has been reported by several investigators

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[1] *J. Ind. Eng. Chem.*, **5**, 650 (1913).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, **21**, 63 (1938).

(Paloheimo, *Biochem. Z.*, **214,** 161 (1929); Norman and Jenkins, *Nature,* **131,** 729 (1933); *Biochem. J.*, **28,** 2147 (1934); and Hilpert and Littman, *Ber.*, **67,** 1551 (1934); **68,** 16 (1935)) that strong mineral acids, such as are employed in the determination of lignin, react with certain carbohydrates with the formation of insoluble humin-like materials, it seemed of importance to determine whether under the conditions prescribed by the method of Goss and Phillips, *This Journal,* **19,** 341 (1936), which has been adopted as a tentative method by this Association, such insoluble products are obtained. The results obtained are given in detail in a paper published in *This Journal,* **21,** 140 (1938). They may be summarized as follows:

(1) Xylose, arabinose, glucose, mannose, galactose, maltose, starch, and cellulose (alone or in the presence of lignified cellulosic material), when treated with fuming and dilute hydrochloric acid in accordance with the procedure of Goss and Phillips for the quantitative estimation of lignin, do *not* afford insoluble humin-like precipitates and therefore would *not* interfere with the determination of lignin in plant materials containing these carbohydrates.

(2) Insoluble humin-like materials were obtained only in the case of sucrose, fructose, and inulin. However, the method of Goss and Phillips calls for a preliminary extraction of the plant materials with hot water and one per cent hydrochloric acid, so that these carbohydrates would be removed by this treatment, and would therefore not interfere appreciably with the determination. It may, therefore, be concluded that in the determination of lignin by the method of Goss and Phillips, the carbohydrates generally associated with lignin in the plant do not interfere appreciably with the determination.

It is recommended[1] that studies be continued on the quantitative estimation of lignin.

REPORT ON ENZYMES

By A. K. Balls (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

There was no work done on pepsin. A large amount of research is necessary first to establish the best type of reaction on which to base a pepsin assay. The Referee considers that it would be better to defer this work until it can be done in connection with some other problem in the laboratory, and not entirely for itself. It is accordingly recommended that this subject be dropped.

The method of Balls, Swenson, and Stuart for the assay of papain was published in *This Journal,* **21,** 97 (1938) and was made the subject of collaborative work as reported in 1935, *Ibid.*, **18,** 140 (1935). Since

[1] For report of Subcommittee A and action by the Association, see *This Journal,* **21,** 64 (1938).

that time the method has had some use by various analysts in the industries, the Food and Drug Administration, and the Experiment Station of Hawaii. It is recommended[1] that this method be tentatively adopted. This action should facilitate further study, as well as make a record of a method that, though probably imperfect, is better than no method at all.

The new method of Balls and Hoover for the clotting of milk by papain can not take the place of the titration methods for all purposes, although it may be sutstituted therefor at times. It is thought that the two methods may supplement, rather than compete with each other.

For report on pepsin, see report of the Referee on Enzymes.

No report on papain was given by the associate referee. See report of the Referee on Enzymes.

A paper, entitled "Activity of Lipase at Low Temperatures," was presented by Balls and Tucker.

A paper, entitled "Amylase of Virus-infected Tobacco," was presented by Balls and Martin.

The paper, entitled "Method for Determining Egg Quality," presented by Sam R. Hoover, appears in this number of *This Journal*, page 496.

REPORT ON WATERS, BRINE, AND SALT

By A. E. Mix (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

BORON

Since the methods for the determination of boron in water are time-consuming, it is desired to obtain a shorter method for collaborative study. The present official method, *Methods of Analysis, A.O.A.C.*, 1935, 523, depends on the distillation of boron in the presence of methyl alcohol, and it is generally recognized that methyl alcohol is an excellent agent to produce volatilization of boric acid.

Good results were obtained by the Cook-Wilson[2] method, which requires 4 hours for completion. In an effort to shorten this time, varying amounts of methyl alcohol were added to the acidified borax solution, and it was found that the pink color developed on the turmeric paper strips in less than 1 hour. A solution containing 30 cc. of water, 20 cc. of methyl alcohol, and 5 cc. of concentrated hydrochloric acid produced the

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 63 (1938).
[2] *J. Agr. Research*, 10, 594 (1917).

STANDARD STRIP NO.	AMOUNT OF $Na_2B_4O_7 \cdot 10H_2O$ *mg.*	COLLABORATORS			
		A	C	B	D
		Test No. 1, sodium borate solution used			
7	0 0	7	7	7	7
3	0.1	3	3	3	3
9	0.2	9	9	9	9
1	0.3	1	1	1	1
5	0 4	5	5	5	5
8	0.5	8	8	8	8
2	0.6	2	2	2	2
4	0.7	4	4	4	4
6	0.8	6	6	6	6
11	0.9	11	11	11	11
12	1.0	12	12	12	12
10	1.1	10	10	10	10
		*Test No. 2, boric acid solution used**			
5	0.0	5	5	5	5
4	0.1	4	4	4	4
6	0.2	6	6	6	6
3	0.3	2	2–3†	2–3†	2
2	0.4	3	3	3	3
7	0.5	7	7	7	7
8	0.6	8	8	8	8
9	0.7	9	9	9	9
1	0.8	1	1	1	1
10	0.9	10	10	10	10
11	1.0	11	11	11	11
		Test No. 3, boric acid solution used‡			
9	0.0	9	9	9	9
8	0.1	8	8	8	8
7	0.2	7	7	7	7
4	0.3	4	4	4	4
1	0.4	1	1	1	1
2	0.5	2	2	2	2
3	0.6	3	3	3	3
5	0.7	5	5	5	5
6	0.8	6	6	6	6
11	0.9	11	11–10	10–11	10–11
10	1.0	10	10–11	11–10	11–10

* Boric acid used in Tests 2 and 3 expressed in mg. of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) in order to compare with Test 1.
† Uncertain as turmeric strip used was slightly faded.
‡ Boric acid solution was placed in 10 cc. of concentrated Na_2MgSO_4, water (1.2 grams solids in solution).

most definite color zone. This solution was used in Tests 1 and 2; in Test 3 the solution used contained 20, 25, and 5 cc., respectively, of water, methyl alcohol, and acid. Upon this basis the following procedure was devised:

Into each of several porcelain casseroles (150 cc. capacity), place 30 cc. of sample, 20 cc. of methyl alcohol, and 5 cc. of concentrated HCl, and mix well by stirring. Also prepare a series of standard borax solutions in the same manner. Each casserole should contain 50–55 cc. of solution. Place the casseroles on the base of a 6-funnel filter rack and fasten one end of each turmeric strip (10 inches long and ½ inch wide) to the upper edge of the filter rack. Have all strips exactly the same length so that the adjustment of the filter rack will allow ½ inch of the bottom end of the strips to dip into the center of the liquid in each casserole. Allow the strips to stand until the maximum color develops (1 hour usually). Protect the strips from direct air currents and do not allow them to adhere to the sides of the casseroles. Remove the strips from the filter rack and compare the intensity and the width of the red color zone with the standards while the strips are moist.

The Referee prepared the solutions and the strips of turmeric paper. The collaborators matched the strips and evaluated the colors. The results are shown in the preceding table.

The collaborators also read the reverse side of the turmeric strips and obtained identical results.

CONCLUSIONS

The results obtained show that the method presented is applicable to the determination of small quantities of borax in mineral waters.

The presence of large quantities of sodium and magnesium sulfates does not interfere with the color reaction.

Sodium phosphate added to one sample did not interfere with the color reaction.

Several colorimetric solutions were produced by a slight variation of this method.

RECOMMENDATIONS[1]

It is recommended—

(1) That further study be made of the method presented, also of a method producing red colored solutions that may be read by means of the neutral wedge photometer described by Clifford and Wichmann, *This Journal*, **19**, 130 (1936).

(2) That collaborative work be done on these methods.

(3) That the following changes be made in the methods for the analysis of waters, brine, and salt in *Methods of Analysis, A.O.A.C.*, 1935: In sec. **14 (c)**, p. 506, "0.0001 mg of N as NO_2" in the 4th line be changed to "0.0001 mg of N." In sec. **16 (b)**, p. 506, "1 cc = 0.01 mg of N as NO_3" in the 6th line be changed to "1 cc = 0.01 mg of N."[2]

No report on mineral salts and effervescent salts was given by the associate referee.

No report on dairy products was given by the referee.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, **21**, 73 (1938).
[2] Action of the Association approving this recommendation was omitted in the report of Subcommittee D, *This Journal*, **21**, 73 (1938).

REPORT ON BUTTER

PREPARATION OF SAMPLE AND DETERMINATION OF FAT

By Roy S. Pruitt (U. S. Food and Drug Administration, New Orleans, La.), *Associate Referee*

As now written the methods for preparation of butter sample and determination of fat are not specific in regard to some of the operations. For example, in the method for preparation of butter sample, the range of temperature at which the sample should be softened is not given although it is obvious that it should be softened sufficiently to mix well. The temperature used should not be too high, or the fat will separate from the water and curd and make it practically impossible to obtain results that will check. The method for the determination of fat does not define clearly the amount of washing that is necessary to remove the fat from the non-fat solids. This washing operation is very important, since failure to remove the fat from the non-fat solids will cause a correspondingly lower fat percentage to be reported. It is, however, a waste of time and material to wash more than is necessary.

The work on butter this year was confined to a study of these deficiencies in the two methods in order to define (1) the temperature at which butter samples should be softened in order to obtain a homogeneous sample, and (2) the amount of washing necessary to remove the fat from the non-fat solids.

It was impossible to submit check samples to the collaborators, as this would have necessitated thorough mixing before they were sent out. Each analyst was requested to work on samples typical of those usually encountered, and not on those already mixed.

In order to have the work cover as many types of butter under as many different conditions as possible, the study was made on samples collected by the New Orleans Station in its regulatory work. These samples represent the product of six creameries in widely separated areas. All the collaborators (W. H. King, J. H. Watkins and J. P. Aumer) had had considerable experience in the examination of butter. They were instructed to soften the sample at the lowest temperature at which the butter could easily be mixed. The instructions given for the washing of the non-fat solids included transfer of the dried sample to a prepared Gooch crucible with 50 cc. of petroleum ether, and washing with 25 cc. portions until the loss in weight was negligible. The detailed methods were published in *This Journal*, **21**, 84 (1938).

The work conducted by the Associate Referee and the results obtained are shown in the tables.

Table 1 shows the temperature that the collaborator found to be the best for mixing each individual sample. Samples 18 and 24 were heated to such a degree that the fat separated from the water and curd. Duplicate

TABLE 1.—*Optimum mixing temperatures*

SAMPLE NO.	MOISTURE	NON-FAT SOLIDS	FAT	MIXING TEMPERATURE
	per cent	*per cent*	*per cent*	°C.
1	16.90	3.16	79.94	36
2	15.67	3.01	81.32	35
3	16.88	3.00	80.12	35
4	16.51	3.17	80.32	35
5	16.61	3.19	80.20	36
6	16.15	3.10	80.75	36
7	16.26	3.67	80.07	36
8	15.72	2.93	81.35	36
9	16.69	3.30	80.01	36
10	15.43 15.45	3.48 3.41	81.09 81.14	36
11	15.65 15.65	3.55 3.51	80.80 80.84	36
12	15.00 14.99	3.62 3.59	81.38 81.42	36
13	14.85 14.81	3.62 3.66	81.53 81.53	36
14	15.15 15.18	3.72 3.76	81.13 81.06	36
15	14.11 14.14	3.35 3.33	82.54 82.53	36
16	15.04 15.03	3.64 3.65	81.32 81.32	36
17	15.00 14.99	3.54 3.56	81.46 81.45	36
18	14.98 14.72	3.50 3.19	81.52 82.09	41
19	15.01 14.97	3.60 3.91	81.39 81.12	37
20	15.09 14.95	3.34 3.33	81.57 81.72	37
21	15.20 15.14	3.49 3.61	81.31 81.25	36
22	15.26 15.27	3.51 3.64	81.23 81.09	36
23	15.42 15.44*	3.95 3.84*	80.63 80.72*	37
24	14.65 14.83*	3.76 3.85*	81.59 81.32*	39.5
25	15.26 15.19*	3.96 4.01*	80.78 80.80*	37
26	15.38 15.36*	3.85 3.88*	80.77 80.76*	36
27	15.22 15.22*	3.99 4.00*	80.79 80.78*	36

* Sample shaken 2 minutes instead of 1 in preparation.

results on these two samples do not agree, and in each case the analyst reported difficulty in obtaining a homogeneous sample. The samples (except those marked) were shaken one minute in preparation. This shaking time seems to be sufficient as duplicate results agree very closely.

TABLE 2.—*Removal of fat by washing*

SAMPLE NO.	LOSS IN WEIGHT, SECOND WASHING	LOSS IN WEIGHT, THIRD WASHING	SAMPLE NO.	LOSS IN WEIGHT, SECOND WASHING	LOSS IN WEIGHT, THIRD WASHING
	mg.	*mg.*		*mg.*	*mg.*
1	.4	.0	17	.4	.0
2	.5	.0		.4	.0
3	.6	.0			
4	.4	.0	18	3.9	2.3
5	.0	.0		5.2	.0
6	.0	.0			
7	.0	.0	19	4.3	.0
8	.5	.0		3.3	.2
9	1.3	.0			
			20	3.3	.4
10	.0	.0		4.6	.0
	.6	.0			
			21	3.6	.7
11	.6	.0		1.3	.0
	.2	.0			
			22	6.1	.4
12	1.1	.2		4.5	.3
	.7	.2			
			23	.4	.0
13	.9	.0		1.1	.2
	.4	.0			
			24	.5	.1
14	.7	.0		.8	.4
	.2	.0			
			25	.8	.0
15	.3	.0		.6	.0
	.4	.0			
			26	.9	.
16	.0	.0		2.1	.6
	.5	.0			
			27	.3	.2
				.2	.2

The results in Table 2 are those of samples transferred from the drying dish to the Gooch crucible with 50 cc. of petroleum ether and washed with 25 cc. portions. The loss in weight of the non-fat solids after each washing is shown. Sample 18, which showed the greatest loss after the third washing, was one that the analyst heated to such a degree that the fat separated from the water and curd. The collaborator reported difficulty in transferring this sample from the dish to the Gooch.

There was much speculation among the analysts about the possible loss in moisture during the preparation and analysis of the sample. It is impossible to measure the loss caused by the first preparation as there is no way of knowing the moisture content until after the sample has been analyzed. Table 3 shows the loss in moisture during the first and second preparation of samples when the method as clarified by the Associate Referee is followed, *loc. cit.* After being prepared and analyzed the samples

TABLE 3.—*Preparation of sample*

SAMPLE NO.	FIRST ANALYSIS			ANALYSIS OF REPREPARED SAMPLE			FAT EXCESS 2ND ANALYSIS OVER 1ST
	MOISTURE	NON-FAT SOLIDS	FAT	MOISTURE	NON-FAT SOLIDS	FAT	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
28	16.96	3.90	79.14	16.94	3.75	79.31	+.17
29	17.60	3.75	78.65	17.55	3.74	78.71	+.06
30	16.87	3.68	79.45	16.98	3.57	79.45	.00
31	16.63	4.45	78.92	16.63	4.34	79.03	+.11
32	16.96	3.90	79.14	16.99	3.71	79.30	+.16
33	16.50	4.62	78.88	16.60	4.55	78.85	−.03
34	16.45	4.33	79.22	16.42	4.38	79.23	+.01
35	15.74	4.85	79.41	15.81	4.76	79.43	+.02
36	15.74	4.87	79.39	15.78	4.80	79.44	+.05
37	16.07	4.87	79.06	16.11	4.70	79.19	+.13
38	16.14	4.85	79.01	16.22	4.69	79.09	+.08
39	15.89	4.61	79.50	15.92	4.53	79.55	+.05
40	16.04	4.58	79.38	16.08	4.49	79.43	+.05
41	16.66	4.15	79.19	16.80	3.98	79.22	+.03
42	17.42	4.36	78.22	17.46	4.29	78.25	+.03
43	17.47	4.59	77.94	17.48	4.50	78.02	+.08
44	16.77	3.75	79.48	16.80	3.74	79.46	−.02
45	17.23	3.88	78.89	17.16	3.86	78.98	+.09
46	17.01	3.79	79.20	16.90	3.80	79.30	+.10
47	16.13	4.55	79.32	16.09	4.73	79.18	−.14
48	18.46	3.74	77.80	18.40	3.75	77.85	+.05
49	18.37	3.58	78.05	18.34	3.60	78.06	+.01

were placed in an ice box at a temperature of 40°F. for 48 hours and were then again prepared and analyzed by a different analyst. The results show that the difference between the first and second analyses is within the experimental error of the method.

DISCUSSION

It was not the purpose of this work to effect any material changes in the methods as they are now written for preparation of butter samples and for determination of fat, but it was found in this study that below 34° C. the butter is not soft enough to be easily mixed, and that above 39° C. the fat separates from the curd and water. The best temperature for preparation of sample would therefore be between 34° C. and 39° C. Table 4

shows that two analysts are able to duplicate results very closely when the samples are prepared by the suggested method. It is recommended, therefore, that the tentative method for preparation of butter samples be clarified by the insertion of a specification that the samples shall be softened to a temperature of between 34° and 39° C.

The study of the amount of washing necessary to remove the fat from the non-fat solids shows that 100 cc. of petroleum ether properly used is sufficient. The following scheme of washing was found to be very satisfactory:

Macerate the dried sample in the drying dish with 15–20 cc. of ether. Transfer the non-fat solids to the prepared Gooch crucible with 30–35 cc. of the ether. Wash the non-fat solids with two 25 cc. portions of ether, taking care not to apply too much suction during the filtration.

In some sections of the country where the relative humidity is rather high, it has been found necessary to take extra precautions not to allow the ether to evaporate too fast in order to prohibit the condensation of moisture, which dissolves part of the salt. It is recommended[1] that the tentative method for the determination of butterfat by the indirect method be clarified by the insertion into the method of a statement to the effect that the non-fat solids shall be washed with 100 cc. of ether after the sample is macerated in the drying dish with 15 cc. of ether. It is also suggested, as a caution, that the non-fat solids be washed with 25 cc. portions of ether until there is no further loss in weight.

REPORT ON CHEESE

By Carl B. Stone (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

Last year Subcommittee C suggested a study of methods of isolating fat from cheese for the determination of fat properties and constants, with special reference to such treatments as promised a minimum change in properties of the fat.

The Associate Referee was not able to do enough work on this subject to justify a report at this time, but desires to make the following recommendations:[2]

(1) That the tentative method for the determination of total ash in cheese be made official (final action).

(2) That the tentative method for the determination of total chlorides in cheese, as revised, *This Journal*, **20**, 340 (1937), be made official (final action).

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 69 (1938).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 68 (1938).

REPORT ON MALTED MILK

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was recommended that the determination of fat and Reichert-Meissl value by the method proposed by the Associate Referee, *This Journal,* **18,** 454 (1935), be studied collaboratively. Accordingly two samples, one a malted milk, and the other a chocolate-flavored malted milk, were submitted to collaborators. The results are given in Table 1.

TABLE 1.—*Collaborative results*

COLLABORATOR	MALTED MILK	CHOCOLATE-FLAVORED MALTED MILK	
	FAT (10 g. SAMPLE)	FAT (50 g. SAMPLE)	REICHERT-MEISSL VALUE
	per cent	*per cent*	
	8.17	4.91	18.9
1		4.88	
		4.96	
	8.13	4.89	18.6
	8.59	5.03	20.3
2	8.66	5.03	
	8.63	5.00	
	8.66	4.99	20.1
	7.90	5.11	19.3
3		4.91	
		4.96	
	7.90	5.04	19.6
	8.01	4.88	22.5
4		4.89	
		5.22	
	8.17	5.23	21.9
	8.02	5.20	—
5		5.16	
		5.09	
	8.31	5.12	—

While correlation between the results of the various collaborators is not entirely satisfactory, the checks of the individual collaborators agree fairly well, which indicates that the procedure followed was not uniform.

It is interesting to note that the results of the fat determinations on the chocolate-flavored malted milk are better than those on malted milk, despite the fact that a larger quantity of the former was used, and more particularly because the chocolate-flavored product contained more insoluble solids.

The manner of drying the fat suggested in the instructions is believed to be preferable to that of drying in the water oven. When the petroleum ether vapors are removed in the manner described the fat is in constant motion, and therefore the removal of the petroleum ether and of any moisture that may be present is facilitated. It was found that by proceeding in this manner, the time necessary for drying the relatively large quantity of fat obtained is greatly shortened, and the opportunity for possible oxidation is thereby decreased.

It is of the utmost importance that during hydrolysis with acid and in the preliminary drying of the filter-cel cake on the steam bath the material be stirred at frequent intervals. This is necessary particularly in the latter step, since the material has a tendency to cake. For the extraction with petroleum ether the material should, of course, be in a finely divided state and be practically free of moisture. Therefore in the instructions sent out it was recommended that the drying of the filter-cel cake be completed in the water oven.

In the instructions 50 cc. of petroleum was recommended for the extractions. The amount of insoluble solids in products of this nature varies widely. In some instances more ether is necessary in order to secure proper extraction of the fat.

The Associate Referee has received several communications regarding the use of paraffin in these determinations. Collaborator Yakowitz reports that better and less troublesome extractions are obtained by using Parawax. This collaborator also suggested the use of a No. 4 Jena fritted-glass Büchner funnel for collecting and washing the filter-cel cake, and that for the extractions the Knorr tube be fitted with a fused-in fritted-glass filter disc.

One of the most serious errors may be caused by the manner in which the fat is weighed, particularly in the case of small quantities. The best procedure for obtaining the weight of fat (use of counterpoise, cooling in a desiccator or in air, and the length of time of standing before weighing) has not been sufficiently investigated. It is suggested at this time that the fat be weighed with a counterpoise after both flasks have been permitted to stand under atmospheric conditions on the balance table for 20 minutes.

It is the intention of the Associate Referee to submit the method to another collaborative test. In the instructions for conducting the determination the points that have just been discussed will be embodied in the text of the procedure in such a manner that there can be no doubt in the minds of the collaborators as to the method to be followed.

Regarding the results on the determination of the Reichert-Meissl value on the chocolate-flavored malted milk, it will be noted that here, too, as in the case of the fat determinations, the checks of the individual collaborators are satisfactory. In all the work on malted milk thus far

undertaken, the Reichert-Meissl value has been used as an index for judging the representative character of the fat extracted. Since the check results on the Reichert-Meissl values obtained by the different collaborators agree fairly well, it may be assumed that the fat extracted by the method proposed is uniform in composition.

It seems unnecessary to do any further work on the determination of the Reichert-Meissl value itself. This subject has been covered thoroughly and there is no doubt that either steam-bath saponification or glycerol-soda saponification is satisfactory. The Associate Referee believes that steam-bath saponification is preferable, since it requires no blanks.

Appreciation is expressed to M. G. Yakowitz, San Francisco; Marie L. Offut, New York; F. J. McNall, Chicago; and W. O. Winkler, Washington, D. C., all of the Food and Drug Administration, for their splendid cooperation.

RECOMMENDATIONS[1]

It is recommended—

(1) That the suggested method, with the precautions indicated, be further studied.

(2) That no further work be done on the determination of the Reichert-Meissl value.

No report on malted milk, microanalytical methods, was given by the associate referee.

REPORT ON DRIED MILK

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

At the last meeting of the Association it was recommended that methods for the determination of lactic acid be studied collaboratively, and attention was called to a colorimetric method submitted by the Associate Referee for the determination of lactic acid in milk and milk products, *This Journal*, **20**, 130 (1937).

Two samples of dry skim milk were sent out to the following collaborators: I. S. Shupe, St. Louis; F. B. Jones, New York; Manuel Tubis, Philadelphia; Paul A. Mills, Seattle; and Gilbert A. Pitman, San Francisco, all members of the Food and Drug Administration.

The results of this work are given in Table 1.

With the exception of the results reported by Collaborator 4 on Sample 2, the checks of the individual collaborators are satisfactory. Since the actual quantity of lactic acid in these samples was not known, no comment on the percentage returns of the different collaborators can be

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 68 (1938).

made. However, the results are encouraging, and it is believed that with a few changes in the technic of the procedure and also in the text to clarify the meaning better results should be obtained next year.

The results of one collaborator were so entirely out of agreement with the other results reported that they are not given in the table. They vary on Sample 1 from 0.138 to 0.222 per cent and on Sample 2 from 0.429 to 0.596 per cent.

TABLE 1.—*Collaborative results on lactic acid*

ANALYST	SAMPLE 1	SAMPLE 2
	per cent	*per cent*
1	0.088	0.378
	0.093	0.374
2	0.065	0.325
	0.056	0.336
	0.076	0.321
3	0.061	0.35
	0.061	0.34
4	0.055	0.202
	0.055	0.251
		0.153
		0.208
5	0.062	0.306
	0 063	0.313

While no definite reason can be given for the results of Collaborator 4 on Sample 2, it is quite possible that they are due to incomplete extraction of lactic acid.

The collaborators were instructed to use a filter centering around 460 mu in making the photometer readings. They were also instructed to include a copy of their standard curves with their reports. All the curves submitted were of the same general type as that given in the description of the published method.

One of the collaborators reported difficulty in condensing the ether. He was instructed to use a longer bulb-type condenser, and with this set-up he experienced no further trouble from this source. In the summer months, when the tap water is rather warm, difficulty may be experienced in condensing the ether. It is quite possible that in some instances the extraction may not be complete due to the necessity of slowing down the rate of ether flow because the ether should return to the flask in a steady stream. It is the intention of the Associate Referee to investigate a more effective means of condensing the ether than that provided by tap water. Dry ice may answer this purpose.

An extraction time of two hours is specified in the method. On future work it is the intention to extract the material for three hours in order to obviate possible losses when a slowing down of the ether flow is necessary.

None of the collaborators seemed to experience difficulty with the method. Pitman recommended that in the treatment of the carbon the air be filtered through cotton. This appears to be a good suggestion since the air supply of the laboratory may be contaminated.

It is recommended[1] that study of methods for the determination of lactic acid be continued.

No report on milk proteins was given by the associate referee.

No report on gelatine in milk products was given by the associate referee.

No report on lactose in milk was given by the associate referee.

REPORT ON EXTRANEOUS MATTER IN DAIRY PRODUCTS

By J. D. WILDMAN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Work on this problem was confined to cream and butter. An attempt was made to adapt the methods for the estimation of mold in these products, *This Journal*, **20**, 93 (1937), to use under actual operating conditions. In the case of cream this problem involved the development of apparatus to make it possible to test a large number of samples simultaneously.

The mold estimation method for butter was used during 1937 as a means of studying the effect of season on the occurrence of mold mycelia in sour cream butter and also the effect of careful cream grading on the occurrence of mold mycelia in such butter. It was found that when the cream was separated by careful grading into two lots, one of which was good sour cream and the other off-flavored cream, the highest mold mycelia count on the butter churned from the good cream was 16 per cent of the fields, while the counts on the butter made from the off-flavored cream varied from 66 to 100 per cent.

No report on decomposition in dairy products was given by the associate referee.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 68 (1938).

REPORT ON NEUTRALIZERS IN DAIRY PRODUCTS

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

At the last meeting of the Association the subject of neutralizers in dairy products was recommended for study. In the present report the investigation is restricted to skim milk powders. After careful consideration the Associate Referee decided to use determinations of lactic acid, titratable acidity, volatile acidity, and the alkalinities of the ash for interpreting neutralization.

It is well established by Hillig, *This Journal*, **20**, 130 (1937), that lactic acid is present in appreciable quantity in milks of poor quality, and since fresh milk quite uniformly contains only a few milligrams of lactic acid per 100 grains, this acid should be a deciding factor in the interpretation of the quality of the dried product.

In order to conceal evidence of spoilage (indicated by high acid content) and more particularly to aid in the drying process, it is quite a common practice for manufacturers to resort to neutralization. As neutralized milk will show abnormal alkalinities of the ash, this factor was also considered important in this investigation.

For the determination of acidity 5 grains of the milk was diluted with 100 cc. of water, and titrated with 0.1 N alkali. The determinations of the alkalinities of the ash were made in the usual manner of ashing 5 grains of the milk at 550° C. For the determination of volatile acidity 5 grains of the powder was thoroughly mixed with 50 cc. of water and 5 cc. of normal sulfuric acid was added, followed by 5 cc. of a 20 per cent phosphotungstic acid solution. After being diluted to 100 cc. the mixture was filtered, and 50 cc. acidified with 5 cc. of normal sulfuric acid was taken for steam distillation; 250 cc. of distillate was collected and titrated with 0.01 N alkali, phenol red indicator being used. The lactic acid was determined according to the Hillig method (*loc. cit.*)

The results obtained are shown in the following table.

From the data recorded it is evident that lactic acid is the significant factor in the judging of dried milks, not only for spoilage, but indirectly for the interpretation of neutralization. Milks low in initial acidity but high in lactic acid point to neutralization and the results of this investigation corroborate these observations. It is interesting to note that the alkalinities of the water-soluble ash in the products marked "not neutralized" are low and rather uniform as compared with those marked "neutralized."

Because of the perishable nature of liquid milk, the only practical way to conduct an investigation of this kind would be to perform the necessary work at the time of processing. Such an investigation would be of value since it would determine whether the lactic acid content is

Acidities and alkalinities of ash of dried skim milk

HISTORY	LACTIC ACID	TITRATABLE ACIDITY (0.1 *N* PER 100 g.)	VOLATILE ACIDITY (0.1 *N* PER 100 g.)	ALKALINITY OF WATER-SOLUBLE ASH (0.1 *N* PER 100 g.)	ALKALINITY OF ASH (0.1 *N* PER 100 g.)
	per cent	*cc.*	*cc.*	*cc.*	*cc.*
Unknown	0.051	178	2.5	15.5	718
Unknown	0.053	178	2.4	16.0	728
Not neutralized	0.060	196	2.6	19.0	711
Not neutralized	0.067	195	2.6	17.5	734
Unknown	0.080	174	2.5	16.5	701
Unknown	0.097	192	3.2	14.0	710
Unknown	0.107	190	3.3	15.5	698
Unknown	0.108	186	3.0	15.0	701
Not neutralized	0.108	194	2.4	16.0	704
Not neutralized	0.118	199	4.2	18.5	707
Not neutralized	0.128	164	3.8	19.5	720
Unknown	0.149	190	3.7	16.5	699
Unknown	0.183	200	4.6	16.0	702
Neutralized	0.240	131	8.1	25.0	773
Not neutralized	0.261	185	6.9	18.0	715
Not neutralized	0.376	204	8.3	15.0	699
Not neutralized	0.408	208	4.1	17.0	711
Not neutralized	0.436	205	6.3	17.0	686
Neutralized	0.497	118	11.6	50.0	859
Neutralized	0.506	53	10.4	61.0	951
Neutralized	0.557	119	10.4	33.0	825
Neutralized	0.812	92	10.5	120.0	914
Not neutralized	1.007	233	20.1	13.5	658
Neutralized	1.297	17	18.2	95.0	1078
Neutralized	1.353	94	21.6	168.0	981
Neutralized	2.012	212	28.9	40.0	863

changed during manufacture. Furthermore, since the data here recorded show an appreciable quantity of volatile acidity in dried milk of questionable quality, the composition of the volatile acidity should be determined.

It is recommended[1] that further study of this subject be made.

REPORT ON TESTS FOR PASTEURIZATION OF DAIRY PRODUCTS

THE PHOSPHATASE TEST IN THE EXAMINATION OF MILK AND CREAM

By F. W. Gilcreas (Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.), *Associate Referee*

Methods for the measurement of the progressive inactivation of enzymes naturally present in fresh milk by heating the product to the

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 68 (1937).

time and temperature of pasteurization have been studied by various investigators with a view to establishing a simple and accurate laboratory test for the control of pasteurization.

Leahy presented one of the first tests.[1] Kay and Graham[2] described a procedure based on the inactivation of the enzyme phosphatase. During incubation this enzyme hydrolyzes an added phenyl-phosphoric ester, liberating phenol, which is readily determined quantitatively by the use of Folin's reagent. Their results indicate a high degree of precision for the method in the detection of minor variations in pasteurizing treatment. Gilcreas and Davis[3] developed a modification of the Kay-Graham technic that distinguishes variations of 5 minutes in time of heating at 143° F., or the lowering of the temperature of pasteurization by 1° F., and in which the presence of 0.1 per cent or more of added raw milk gives a reaction indicative of underpasteurization.

In order to determine the precision with which this test, now adopted as a tentative standard method by the Association of Official Agricultural Chemists,[4] could be used by many different workers, the following laboratories were asked, beginning October 18, 1937, to examine 6 weekly series of 12 samples each.

Bureau of Milk Sanitation, New York State Department of Health, Albany (Mobile Laboratories, Nos. 1 and 2), W. D. Tiedeman, Chief.

Tompkins County Laboratory, Memorial Hospital, Ithaca, New York, B. F. Hauenstein, Director.

New York State Agricultural Experiment Station, Geneva, J. C. Hening.

Bureau of Laboratories, Connecticut State Department of Health, Hartford, F. L. Mickle, Director.

South Dakota State Chemical Laboratory, Vermillion, Guy Frary, State Chemist.

Division of Laboratories and Sanitation, Jacksonville City Department of Health, Jacksonville, Fla., H. N. Parker, Director.

City Health Department, Chicago, Ill., Herman N. Bundesen, President of the Board.

Sealtest Laboratories, Baltimore, Md., Jas. J. Johnson.

Department of Dairy Technology, Ohio State University, Columbus, L. H. Burgwald.

Office of Milk Investigators, U. S. Public Health Service, Washington, D. C., Leslie C. Frank.

Food Research Division, U. S. Department of Agriculture, Washington, D. C., F. C. Blanck, in charge.

Some of the samples, collected from a commercial pasteurizing plant, represented various degrees of pasteurizing treatment, and some were pasteurized milk or cream to which had been added small quantities of raw milk. All the samples were packed with sufficient dry ice to provide

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[1] 23rd Ann. Rept. Intern Assoc. Dairy and Milk Inspectors, 1934, pp. 93-108.
[2] *J. Dairy Research*, **6**, 191 (1935)
[3] 25th Ann. Rept. Intern Assoc. Milk Sanitarians, 1936, pp 15–39; 11th Ann. Rept N. Y State Assoc. Dairy and Milk Inspectors, 1937, pp 83–96
[4] *This Journal*, **21**, 82 (1938).

six hours' refrigeration, without freezing, and were shipped for delivery within 24 hours by parcel post, special delivery, or by air express to the more distant laboratories. Each sample was identified by a serial number only. Previous studies by Gilcreas[1] indicated that storage of milk, un-iced, for 24 hours would not affect the accuracy of the test.

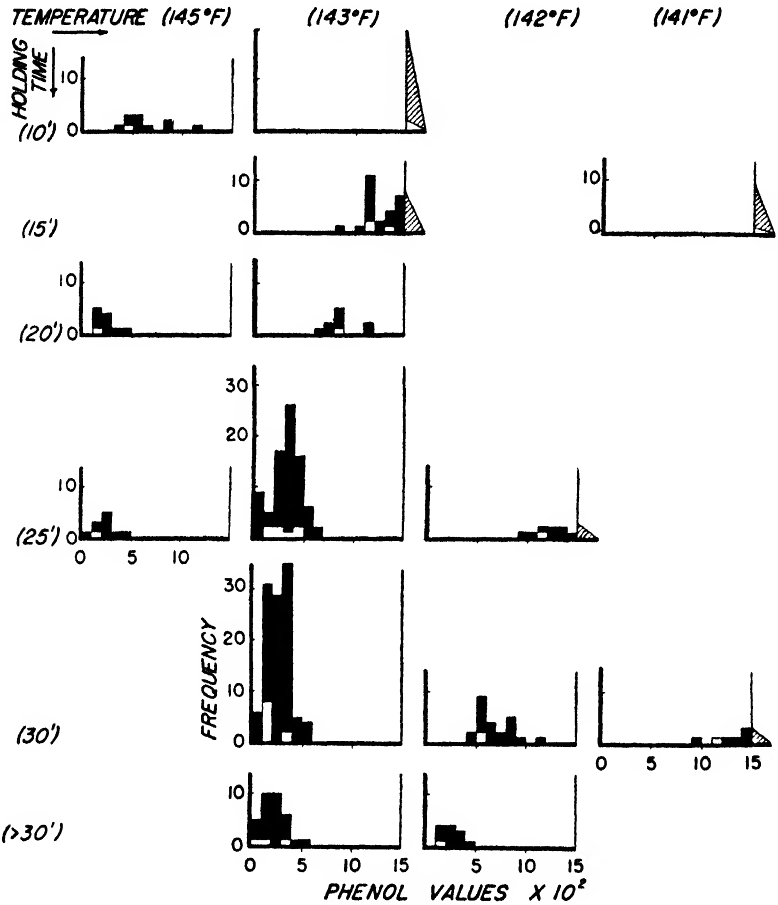

FIG. 1.—FREQUENCY-DISTRIBUTION OF OBSERVED PHENOL VALUES (EXPRESSED AS MG. PER 0.5 ML. OF SAMPLE) FOR MILK HEATED FOR THE GIVEN TIME AT THE GIVEN TEMPERATURE.

Frequency of values >0.15 proportional to area of indicated triangle. Referee values correspond to unshaded portion of diagram.

Results of the examinations as reported by all the laboratories are given in Table 1, which indicates the total number of samples and examinations of each type of sample and the frequency distribution of the results.

[1] *Loc. cit.*

TABLE 1.—*Frequency-distribution of results of analyses (x) by cooperating laboratories*

CHARACTER OF SAMPLE	NUMBER OF SAMPLES	N NUMBER OF ANALYSES	Mg. OF PHENOL PER 0.5 ml. SAMPLE															
			$x>.00$	.01	02	03	.04	.05	.06	.07	08	09	.10	.11	.12	13	.14	.15
			$x\leq$ 01	.02	.03	.04	.05	06	07	.08	.09	.10	.11	.12	.13	14	.15	
Raw Milk	8	80																80
Milk—143° F.																		
Min. holding																		
5	2	21																21
10	2	20																20
15	3	34									1	0	1	11	2	4	7	8
20	1	10						1	1	2	5	0	0	2				
25	7	81	9	5	17	26	16	6	2									
30	10	110	6	31	29	35	5	4										
>30	3	33	5	10	10	6	1	1										
Milk—145° F.																		
Min. holding																		
10	1	11				1	3	3	1		2			1				
20	1	11		5	4	1	1											
25	1	11	1	3	5	1	1											
Milk—143–141° F.																		
Min. holding																		
30	1	11				1	1	4	2	2	1							
Milk—142° F.																		
Min. holding																		
25	1	12										1	1	2	2	2	1	3
30	2	24					2	9	4	2	5	1	0	1				
>30	1	12		4	4	3	1											
Milk—141° F.																		
Min. holding																		
15	1	10																10
30	1	10										1		1	1	1	3	3
Milk—135° F.																		
Min. holding																		
30	1	11																11
>30	1	11																11
Pasteurized Milk with—																		
5% Raw added	1	10																10
0.1% Raw added	3	34			3	10	10	8	1	2								
0.05% Raw added	2	21			3	10	6	2										
Light Cream Raw	2	18																18
Pasteurized Light Cream with—																		
10% Raw added	1	11																11
5% Raw added	1	10																10
1% Raw added	2	21									1							20
0.5% Raw added	1	12															2	11
0.1% Raw added	5	55		1	6	9	14	17	1	4	2							1
0.0% Raw added	3	33	4	11	12	5	1											
Pasteurized Heavy Cream with—																		
0.1% Raw added	2	24				2	3	10	3	4	2							
0.0% Raw added	1	12	2	3	6	1												
Totals	72	784	27	73	99	111	65	64	15	16	19	3	2	18	5	7	13	248

A phenol value of 0.04 mg. per 0.5 ml. of milk had been established by Gilcreas[1] as indicative of complete pasteurization at 143° F. However, further experience indicated that variations in time of cooling or pre-

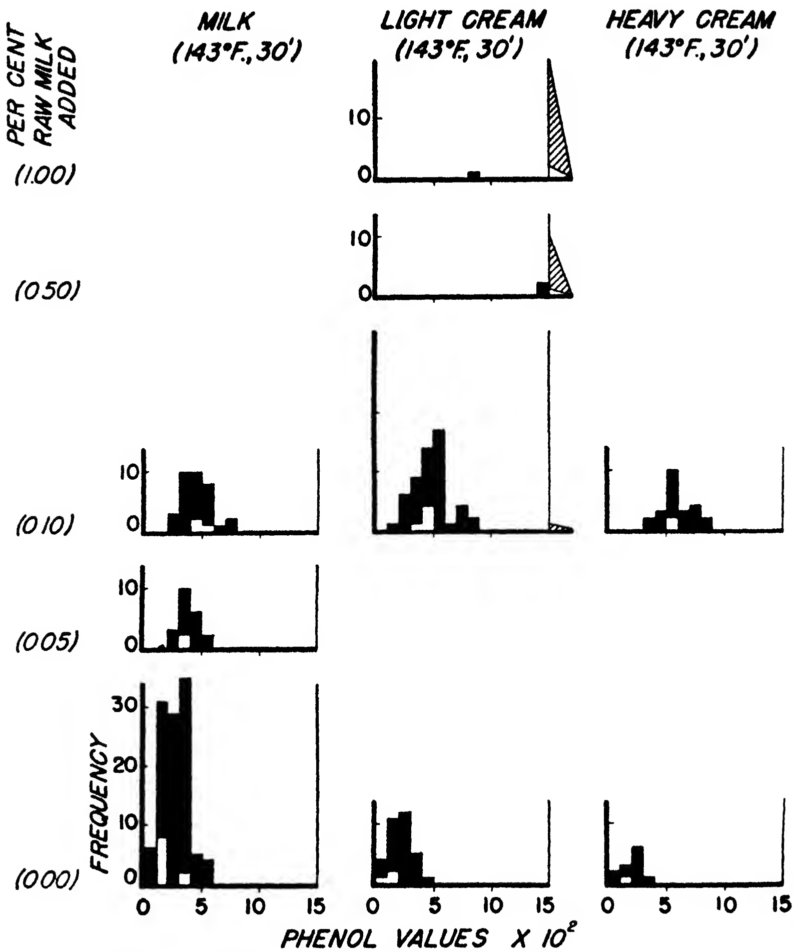

FIG. 2.—FREQUENCY-DISTRIBUTION OF OBSERVED PHENOL VALUES (EXPRESSED AS MG. PER 0.5 ML. OF SAMPLE) FOR MIXTURES OF RAW MILK AND PASTEURIZED MILK OR CREAM.

Frequency of values >0.15 proportional to area of indicated triangle. Referee values correspond to unshaded portions of diagrams.

heating might cause slight variations in this value, and accordingly a phenol value of <0.05 mg. per 0.5 ml. of milk was selected in this evaluation as an indication of complete treatment at this temperature.

Fig. 1 shows graphically the frequency-distribution of results obtained with milk heated to the given temperature and for the given periods.

[1] *Loc. cit.*

Frequencies are plotted as ordinates and phenol values as abscissas. The diagrams indicate the results of all laboratories and the unshaded portions those of the referee laboratory. In Fig. 2 are shown the results of analyses of samples of pasteurized milk and cream to which varying proportions of unheated milk had been added.

Examination of the results shows that of 110 samples that had been heated to 143° F. and held for 30 minutes, 104 were reported to have phenol values less than 0.05 mg. per 0.5 ml. Therefore, pasteurized milk was detected with a precision of 96 per cent.

Operating variations in the pasteurizing plant from which the samples used in this investigation were obtained frequently extend the time of preheating. Thus, in many cases portions of milk collected after a recorded 25-minute holding period represent an actual time of heating equivalent to 30 minutes or longer. This factor probably explains the failure to detect technical holding periods of 25 minutes with the anticipated precision. Addition of raw milk in an amount as low as 0.1 per cent was detected in 50 per cent of the samples examined. Instances of the addition of raw milk in larger amounts and heating to 143° F. for 20 minutes or less were detected without exception.

The cooperating laboratories were asked to make a control test on each sample examined. In the few instances where the recommended technic for the control test was not followed in detail, the resulting phenol values were rejected. The remaining results on milk heated to 143° F. for the indicated holding periods, compiled in Table 2, show that the control

TABLE 2.—*Frequency-distribution of results of control tests (x_0) by cooperating laboratories*

CHARACTER OF SAMPLE	NUMBER OF SAMPLES	N NUMBER OF ANALYSES	Mg. PHENOL PER 0.5 ml. SAMPLE				
			$x_0 > .000$	.010	.020	.030	.040
			$x_0 \leq 010$	.020	.030	.040	.050
Raw Milk	8	70	34	25	8	3	0
Milk heated to 143° F., held 5–25 minutes	15	143	87	43	9	2	2
Heated to 143° F., held 30 minutes	10	92	61	24	5	2	0
Totals	33	305	182	92	22	7	2

value was practically constant at from 0.01 to 0.02 mg. per 0.5 ml. of sample. The close agreement obtained justifies elimination of the control test in the examination of all samples except those found to be underpasteurized. A control value distinctly higher than 0.02 would indicate the possible presence of interfering substances, and the excess should be

subtracted from the value found on the examination of the sample in order to determine the phenol concentration indicative of pasteurization treatment.

The use of a temperature of pasteurization below 143° F. would require the establishment of a phenol concentration indicative of complete treatment at that temperature and would also reduce proportionately the sensitivity of the test, particularly in the detection of added raw milk.

Variations in phosphatase content, as reported by Folley and Kay,[1] may limit the sensitivity of the test to detect minor differences in heat treatment.

CONCLUSION

The results of this cooperative study indicate a high degree of precision and sensitivity in the phosphatase test presented. It is recommended[2] that additional studies be made of its precision and practical application with a view to the adoption of a standard method for the determination of pasteurization.

The assistance of W. R. Thompson in the compilation and evaluation of the results of this study was of inestimable value in the preparation of this report. To him is also due the credit for the graphic representation of the results.

REPORT ON NAVAL STORES

By F. P. VEITCH (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

There is a growing interest in methods for the examination for purity and identity of turpentine, rosin, and other products classed under the general term "naval stores." This interest starts primarily in the consuming industries because they want to know that they get materials of the grade, quality, and purity desired or specified, as a means of maintaining the uniform characteristics of their own products in which these materials are used. Of necessity, the interest on the part of consumers brings about a need for knowledge of the characteristics of their products on the part of producers, in order effectively to meet demand and competition.

TURPENTINE

There are four kinds of spirits of turpentine produced in this country today; gum spirits of turpentine constitutes approximately 75 per cent of the total; steam-distilled wood turpentine, approximately 20 per cent; sulfate wood turpentine, approximately 4 per cent; and destructively distilled wood turpentine, approximately 1 per cent. The production of sulfate wood turpentine has been increasing rapidly, owing to the tre-

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[1] *Enzymologie*, 1, 48–54 (1936).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 21, 69 (1938).

mendous increase in production of sulfate wood paper pulp by the paper mills of the South, all of which are using pine for the purpose. Commerical sulfate wood turpentine, a by-product, is made by so refining the crude digester condensate that only a trace of the mercaptans and sulfides present in the crude in considerable quantities remain.

It is because of this growing need for differentiating between these several kinds of spirits of turpentine, and for the recognition of mixtures of them, or adulterations with petroleum or coal tar oils, that a steadily increasing demand for accurate, rapid methods for the examination of turpentine is to be anticipated, not only on the part of industrial purchasers, for their individual needs, but also on the part of those officials charged with the administration of State laws. There is still adulteration and misbranding of spirits of turpentine, especially on the part of small dealers doing intrastate business. The need of methods for determining the color; the iron, copper, zinc, and/or aluminum present; and the suitability of turpentine for special purposes, such as for making synthetic camphor, is growing.

The Referee and his associates are working closely with the Naval Stores Committee of the American Society for Testing Materials and others in the development of necessary methods for the examination of turpentine and rosin.

ROSIN

Of the rosin annually produced in this country, approximately 70 per cent is gum rosin made by simply distilling off the turpentine and water from the oleoresin of longleaf and slash pine as it has exuded from the living tree, and straining out the chips and trash from the remaining molten residue, which is designated "rosin"; the other approximately 30 per cent is wood rosin, made from the chips of pine stumpwood by extracting the rosin with gasoline, after the chipped wood has been steamed to remove turpentine and pine oil. These two types of rosin are easily differentiated by any one who has once examined both kinds by their distinguishing odors when heated. Gum rosin has a characteristic aromatic odor somewhat suggestive of spices and turpentine, and, under present commercial still practice, as generally carried out, most gum rosin contains specks of extraneous matter, such as bark, sand, dirt, and water-soluble matter that gets into the oleoresin during the time it is being collected in the cups hung on the trees. Wood rosin, on the other hand, has a mixed odor suggestive of burnt sugar and pine wood, is free from dirt and specks, and consequently is brilliantly clear. Originally, all wood rosin was dark red in color, and of low grade, —FF or below. Within recent years, however, processes of production have been modified and improved, so that today the larger producers of wood rosin can supply any of the high-grade products, although N grade is the highest quality usually made. These high-grade wood rosins retain the burnt

sugar-pine odor by which they can be differentiated from gum rosins.

In addition to sulfate wood turpentine, there is also being recovered from the waste liquors from cooking pine wood by the sulfate process, a complex mixture of fatty and resin acids, in the form of a heavy dark brown viscous liquid. This product is generally designated in Europe, where it was first recovered, as "tallöl," a Swedish term which means, literally, pine oil. It is sometimes improperly referred to in this country as "liquid rosin." This product contains about 40 per cent of resin acids. The material finds its largest use in this country today in the preparation of adhesives, insecticide and fungicide sprays, and certain types of industrial soaps. However, research is being conducted to develop commercial methods for the separation of the fatty and the resin acids. If this is accomplished, a third type or kind of rosin may result. It will, of course, be a wood rosin, but it will probably differ sufficiently from such article as now prepared as to require a separate classification.

The methods for the determination of acid and saponification numbers of rosin have been subjected to further cooperative study, and it is sufficient to report that they have proved fairly satisfactory. Some trouble is still encountered, due to the apparent attack of the alkali on the digestion flasks during saponification.

W. C. Smith of the Referee's laboratory made a study of the influence of solvents on the saponification number of rosin.[1] This study included saponifications in the following solvents: C. P. methyl alcohol nearly absolute; 95.2 per cent ethyl alcohol distilled over potassium hydroxide; No. 30 specially denatured alcohol (90 per cent by volume ethyl plus 10 per cent methyl alcohol); commercial isopropyl alcohol ("Petrohol") refined; and n-butyl alcohol, technical grade, refined. He found, in harmony with findings of others, that the saponification number of rosin is influenced by the solvent used, the higher alcohols giving the higher values, and that No. 30 denatured alcohol yields essentially the same results as does pure 95 per cent ethyl alcohol. Since a great deal of cooperative work has been done showing that in saponification work No. 30 denatured alcohol may be substituted for 95 per cent ethyl alcohol, it is recommended that the use of No. 30 denatured alcohol be made tentatively alternative with 95 per cent ethyl for the determination of saponification number of rosins.

The methods of the Association for the examination of rosin are satisfactory as far as they go. They should be extended. The growing interest in the use of rosin is developing a need for suitable methods for determining softening point; copper, manganese, and iron content; crystallization tendency; identity of the resin acids present; contamination with mineral oil; etc., because each of these subjects plays an important part in one or

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[1] *Ind. Eng. Chem. Anal. Ed.*, 9, 10 (1937.)

more of the major uses of rosin. Methods for these determinations are now under individual and cooperative study, and will be brought to the attention of the Association for action as soon as they have been sufficiently developed.

Some difficulty has been met in getting members of the Association to devote time to cooperative work on naval stores methods, but with the growing need for such methods it is hoped that more interest can be aroused.

RECOMMENDATIONS[1]

It is recommended—

(1) That the use of No. 30 denatured alcohol in the determination of the saponification number of rosin be made tentative and alternative with 95 per cent ethyl alcohol, and that there be inserted in paragraph 4, "Saponification Number—Tentative," of the existing methods, after the figure "22" in the second line, the words, "or 50 cc of No. 30 denatured alcohol and 20 cc of alcoholic KOH made up with No. 30 denatured alcohol."

(2) That the work on naval stores be continued with a view to providing methods of examination of rosin not now standardized and available.

For report on rosin see the preceding report of the Referee on Naval Stores.

REPORT ON TURPENTINE

By V. E. Grotlisch (Naval Stores Division, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was pointed out last year that one of the large Eastern oil companies had put on the market several new paint and varnish thinners, described in the trade press as hydrogenated naphthas, and sold under a trade name. These thinners were said to be prepared from the light petroleum oils by a catalytic hydrogenation process or similar chemical treatment. They were found to be polymerizable or soluble in fuming sulfuric acid to a large degree. They might therefore be considered as more suitable for adulterating turpentine than the straight mineral spirits type of thinner. It was found that with the present official polymerization test, with 5 per cent of the oil (thinner) added, only 1 per cent of residue was obtained, that is, only about 20 per cent of theoretical, whereas with the ordinary mineral oil adulterant the recovery is approximately 70 per cent or more of theoretical.

Recently the general nature of these first thinners has been somewhat

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 64 (1938).

changed, in that the latest samples received are now even more soluble in sulfuric acid. Furthermore, a large Western oil company has also entered the field with a new line of solvents having similar properties.

Some preliminary test solutions were prepared to contain 5 per cent of the oil and 95 per cent of pure gum turpentine. Absorption spectra of pure gum turpentine and of the adulterated turpentines were photographed by B. A. Brice of this laboratory on a quartz spectrograph, a hydrogen discharge being used as a continuous source of ultra violet radiation. The spectrograms of all the samples under study showed the presence of certain marked absorption bands in the region from 2400 to 3100 Ångstrom, which are not shown by turpentine itself. Since compounds of the nature of benzol or toluol were suspected to be present, 5 per cent solutions, respectively, of benzol, toluol, and commercial xylol in turpentine were also prepared. The exact nature of the xylol, *i.e.*, whether ortho, meta, para, or a mixture, was not indicated by the label on the bottle.

Spectrograms of these known mixtures were then obtained and compared with those for the adulterated samples under study. Two of the adulterated turpentines showed absorption bands similar to and characteristic of those obtained with the toluol-turpentine mixture. Both the Eastern and Western oil sources were represented in these samples. The other adulterated samples (one Eastern and three Western) showed absorption bands similar to and characteristic of the xylol-turpentine mixture. An additional absorption band at another point was also noted with one of the latter samples, due probably to another constituent of a nature similar to xylol, or possibly to one of the xylol isomers not represented by the xylol used to prepare the comparison sample.

It has been suggested that by suitable refinement in the measurement of the absorption intensities, or by comparison of these spectra with those of solutions of different concentrations, it would be possible to determine the proportion of coal tar hydrocarbon (toluol, xylol, etc.) in the adulterant contained in a sample under examination.

To attack the problem from a chemist's viewpoint, however, is probably of more interest to members of this Association. A method for detecting and estimating coal tar hydrocarbons (aromatic or ring compounds) in turpentine was published some years ago by the writer.[1] It is believed that this method may be of use in the problem at hand. Further study along chemical lines is also in order because few laboratories are equipped with suitable quartz spectrographs.

The proposed method suggested for further study is somewhat prolonged, and involves a number of steps. Briefly these may be outlined as follows: First a concentration of the adulterant from a large sample

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[1] *J. Ind. Eng. Chem.*, 13, 9, 791 (1921).

(50 cc.) is effected by removal of some of the terpenes of the turpentine, through conversion of pinene into the crystalline pinene hydrochloride. After filtration a sulfonation is made with concentrated sulfuric acid to polymerize the balance of the terpenes. The aliphatic or saturated mineral oil constituents are recovered from the sulfonation mixture by steam distillation, after which the coal tar or aromatic hydrocarbons may be partly regenerated and recovered by a straight distillation of the sulfonation mixture, the temperature of which is gradually increased by removal of water, until finally frothing prevents further recovery. The nature of the oily portion of this final distillate is proved by a nitration to obtain the characteristic odor of the nitrated ring hydrocarbons.

In view of this outline of the scope and nature of the problem under consideration, and the extent of the preliminary work that is necessary before any collaborative study can be started, no effort has been made to secure volunteers for the work, and no collaborative samples have been distributed.

RECOMMENDATIONS[1]

Since certain new paint and varnish thinners now being marketed by several large petroleum companies undergo polymerization and solution in fuming sulfuric acid to a much greater degree than do the usual thinners of the mineral spirits type, the official polymerization test for detecting and determining mineral oil adulteration of spirits of turpentine would not be entirely dependable should adulteration with such new type petroleum thinners be resorted to.

Preliminary examination of the ultra-violet absorption spectra of samples of turpentine containing definite small quantities of these thinners by means of a quartz spectograph has given information indicating that these new thinners contain appreciable quantities of aromatic or coal tar hydrocarbons, such as toluol and xylol, or constituents closely allied thereto. Further study of spectrographic methods of analysis of turpentine would appear warranted.

Since it also appears possible to apply chemical methods for detecting and determining the extent of adulteration of turpentine with reduced or converted petroleum thinners, it is recommended that further study of the problem of devising additional methods for testing turpentine for adulteration with petroleum oils containing aromatic hydrocarbons be carried out by the Associate Referee on Turpentine before the subject is submitted to collaborative study.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 21, 65 (1938).

REPORT ON PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

STUDY OF ABRASION TEST—NEED FOR FILM OF UNIFORM THICKNESS

By C. S. LADD (Food Commissioner and Chemist, Bismarck, N. D.), *Referee*

Examination of paints and varnishes and other film-forming products in this laboratory has shown that there is a definite need for more reliable methods of producing dried films of uniform and desired thickness if reliable results are to be obtained from tests such as abrasion resistance, flexibility, and drying time.

Various methods and apparatus are used today for producing uniform films, and many difficulties are encountered. Perhaps the most common method is that in which the product is flowed on a panel, which is placed in a vertical position to drain and dry. The film produced is thin at the top and gradually thickens toward the bottom. The portion between the two ends that is of the desired thickness is used for the tests. The objections to this method are (1) the limited amount of film of the correct thickness, (2) the lack of uniformity from side to side, (3) its applicability to free-flowing products only, and (4) its nonapplicability to thicker materials such as outside paints.

A similar film is produced by pouring the product on the center of a panel mounted on a disc rotating at a moderate speed for a definite time. The objections to this method are similar to those ascribed to the method previously mentioned. Also, the more plastic material has a tendency to streak from the center, causing unevenness in the film.

Dipping has been used for obtaining uniform films, the thickness being controlled by the rate of withdrawal of the panel. This method has very limited use, and the difficulty in ascertaining the proper rate of withdrawal is easily seen.

For thicker material perhaps the doctor blade method is the best. The apparatus consists essentially of a straight blade supported above the panel by shims of the thickness of the film desired. The film-forming material is poured onto the panel between the shims and leveled off to the desired thickness by the sliding of the blade, the ends of which are supported on the shims. One manufactured tube style consists of a hollow cylinder with a portion of the rim on each end milled out to the desired depths. The instrument is placed on end on the panel, and the material to be spread is placed in it. It is then moved along the panel, and the film escapes through the milled opening. The film thus produced is quite uniform after drying, but it is much thinner than the original wet film, the shrinkage varying according to the composition of the product. It is obvious, therefore, that in order to produce dried films of the desired

thickness a large number of shims or cylinders with milled rims of various depths would be required. Since the shrinkage of a wet film on drying depends upon the amount of volatile thinner present, the production of a dry film of desired thickness by controlling the thickness of the wet film is a difficult problem.

An instrument for applying films by controlling the volume of material flowed over a given area has been devised in this laboratory. The adjustment for controlling this volume is such that a wet film of any thickness up to 0.005 inch can be prepared. The instrument has not been perfected, but it should provide a means of overcoming many of the difficulties encountered when other methods are used. In order to produce on any product a dry film of the required thickness it is necessary to prepare a preliminary film with the instrument set for a definite thickness of wet film. After the film has dried it is measured, and the ratio of the dry film to the original wet film is obtained. From this ratio it is only a simple calculation to determine the thickness of wet film to apply to give the proper thickness of dry film for testing.

In abrasion resistance tests the correct film thickness cannot be too strongly stressed. The Gardner abrasion test is used in all work done in this laboratory on floor varnishes and enamels. In this test sand is allowed to fall a distance of one meter onto a glass panel supported at an angle of 45° and covered with a dry film of the material under test. The quantity of sand required to wear through a film 25 microns thick is taken as a measure of resistance to abrasion. It was found that small variations in film thickness cause marked differences in the results. It was also found that the relationship of the film thickness to the quantity of sand required in this test is not a constant; the amount of sand necessary to wear through the film is much less in relation to the proportional decrease in film thickness. Hence, in order to expect duplication of results it is essential that the film be uniform, and to obtain comparative values of various products the films must be of equal thickness.

With the Gardner test much difficulty was experienced, especially on films of higher resistance, in obtaining duplicate results, even though the film appeared to be uniform by measurements. The film does not appear to wear away gradually until it disappears; rather, after a partial erosion it begins to shatter and chip off in minute particles. It would appear that the surface of a film is not so readily attacked by the sand at first, due, perhaps, to a cushioning effect of the film between its surfaces and the surface of the panel. This may explain the greater decrease in sand required per decrease in film thickness—the thinner film having less cushioning effect. On films of high resistance much time is required to make this test.

Many methods for determining abrasion resistance have been used, but a relatively simple and reliable method that could be standardized

should be of great importance in evaluating a finish to be subjected to heavy wear.

RECOMMENDATIONS[1]

It is recommended—

(1) That study of the following methods of testing varnishes be continued: Abrasion resistance, hardness, skinning, and alkali resistance, and that an associate referee be appointed.

(2) That study of the accelerated weathering test of paints be continued.

No report on accelerating testing of paints was given by the associate referee.

No report on leathers and tanning materials was given by the referee.

No report on radioactivity was given by the referee.

No report on quantum counter was given by the associate referee.

No report on gamma ray scope was given by the associate referee.

No report on cosmetics was given by the referee.

REPORT ON CEREALS

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

R. M. Sandstedt, has recently published a report on the determination of sucrose in flour.[2] The method is similar in principle to the present official ferricyanide method for the determination of the diastatic activity of flour, *Methods of Analysis, A.O.A.C.*, 1935, 218. It seems desirable for the Association to do some work on a method, or methods, for the determination of sugar in flour, particularly since no collaborative work has been done on the present tentative procedure, *Ibid.*, 209. It is therefore recommended that an associate referee be appointed to study the determination of sugar in flour.

The methods of the Association include a tentative method for the acidity of water extract, but it has received no collaborative study. This method specifies extraction with water and reporting of the acidity as lactic. The Cereal Laboratory Methods Book, published by the American Association of Cereal Chemists, contains two additional methods, the Greek or Balland for the acidity of the alcoholic extract of flour, reported

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 21, 64 (1937).
[2] *Cereal Chem.*, 14, 767 (1937).

as per cent sulfuric acid, and the Schulerud method, which expresses the results as cc. of 0.1 N alkali per 10 grams of flour. The Schulerud method also uses an alcohol and water mixture, but it is of a different strength from that used in the Greek method. Apparently all these methods are used to indicate a certain condition, but they actually measure different constituents. The Grain Division of the Bureau of Agricultural Economics has developed a method suitable for the measurement of the acidity and soundness of grain and grain products. This method may give results of the same interpretative value as that given by any one of these three methods. Accordingly, it is recommended that an associate referee be appointed to study this problem.

The Association has a tentative experimental baking test that is intended primarily for hard wheat flours. Some effort has also been made to develop a similar test for soft wheat flours as the importance of this product seems to justify study in this connection, It is therefore recommended that an associate referee be appointed to direct this work.

As there does not appear to be any immediate need for an official method for the determination of the catalase activity of flour, it is recommended that further work on this subject be discontinued for the present.

RECOMMENDATIONS[1]

It is recommended—

(1) That the work on the development of methods for the measurement of the proteolytic activity of flour be continued.

(2) That further study be made of methods of extraction and determination of alkaloids and other constituents of ergot as a means of determining ergot in rye flour.

(3) That further study be made of methods for the determination of salt-free ash in macaroni and baked products.

(4) That collaborative work be continued on the determination of color in flour.

(5) That the respective associate referees continue the studies on soy flour in foods, on the development of a method for identification of the farinaceous ingredients present in macaroni, on whole wheat flour, and on phosphated flours.

(6) That the methods for water-soluble protein nitrogen precipitable by 40 per cent alcohol (tentative) under flour, page 211, and under macaroni products (official, first action), page 229, be retained in their present status until they can be studied in connection with the work on the determination of egg solids.

(7) That the inaccurate statement on page 216 of *Methods of Analysis, A.O.A.C.*, 1935, "1 cc of the last soln = 0.0001 mg of N as nitrite," be changed to read: "1 cc of the last soln = 0.0001 mg of N."

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 75 (1938).

(8) That the method for the preparation of sample under baked cereal products, page 221, be made official, first action.

(9) That the method for collection and preparation of sample under macaroni products be made official, first action.

The Referee concurs in the recommendations of the associate referees.

REPORT ON ASH IN FLOUR, MACARONI PRODUCTS AND BAKED PRODUCTS

By L. H. Bailey (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In 1936 this Association adopted as tentative, the magnesium acetate method for ashing cereal products. This method was published in *This Journal*, **20**, 69 (1937).

The need for a rapid and at the same time an accurate method for determining ash in cereal products is as great as was the need for a rapid method for determining moisture. In the hands of the Associate Referee this tentative magnesium acetate method has proved to be quite satisfactory.

This year six analysts that were known to have automatically controlled muffle furnaces for making ash determinations were selected for further collaborative work. The materials ashed were flour, bread, and macaroni. The collaborative results are given in the following table:

Collaborative results

COLLABORATOR	FLOUR ASH	BREAD ASH	MACARONI ASH
	per cent	*per cent*	*per cent*
A	0.42	2.85	0.68
	0.44	2.83	0.67
B	0.44	2.91	0.74
	0.44	2.90	0.72
C	0.47	2.68	0.67
	0.47	2.77	0.70
D	0.41	2.65	0.76
	0.40	2.56	0.75
E	0.44	2.65	0.69
	0.44	2.74	0.70
F	0.44	2.79	0.68
	0.45	2.81	0.67

The duplicate results furnished by the collaborators show that in general they secured good agreement in their own ash values for all three

substances—flour, bread, and macaroni. There is some variation in values between the different collaborators, but it is believed that these differences would be minimized with more experience with the method.

The collaborators were John T. Kiester, J. I. Palmore, and V. E. Munsey of the U. S. Food and Drug Administration; H. C. Fellows of the Bureau of Agricultural Economics; and J. Davidson and L. H. Bailey of the Bureau of Chemistry and Soils, all of the U. S. Department of Agriculture.

Harris and Johnson[1] ashed 20 samples representing different grades and classes of flour by the tentative A.O.A.C. method and the Standard A.A.C.C. method, which is the official A.O.A.C. method. As a result of this work they conclude that the magnesium acetate method of ashing flour is convenient and rapid and yields reproducible results, and that it compares favorably with the Standard A.A.C.C. method in accuracy and precision and reduces the time required 5–6 hours.

RECOMMENDATIONS[2]

It is recommended—

(1) That the tentative magnesium acetate method of ashing cereal products be made official (first action).

(2) That the work be continued.

REPORT ON H-ION CONCENTRATION

By George Garnatz (The Kroger Food Foundation, Cincinnati, Ohio), *Associate Referee*

Lack of time prevented investigation of all the recommendations made by the Associate Referee in 1936, *This Journal*, **20**, 355 (1937), and likewise made it undesirable to undertake further collaborative studies. In order that some progress toward the ultimate development of a satisfactory colorimetric method might be made, it was decided to focus attention on those recommendations covering the desirability of using a greater amount of the nitro-phenol indicators and studies on stabilizing the filtered extract and on determining light conditions favorable to differentiating between colors.

CONCENTRATION OF INDICATOR SOLUTIONS

Three concentrations of indicator solutions were used, corresponding to A, that given with the method in the 1936 report; B, twice this concentration; and C, four times this concentration.

The detailed data for each of the indicators, therefore, are given in Table 1.

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[1] *Northwestern Miller*, 191, 51–52 (1937).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 75 (1938).

The para and metanitrophenols dissolved readily in hot distilled water in the concentrations indicated. The gamma 2:5 dinitrophenol did not dissolve completely in hot distilled water in the B and C concentrations. They did, however, dissolve in a 50 per cent by volume alcohol-water solvent, which was used in the preparation of these two particular indicator solutions.

TABLE 1.—*Indicators*

SOLUTION	CONCENTRATION
	Gamma (2:5) dinitrophenol
A	0.05 gr./100 cc. H_2O
B	0.10 gr./100 cc. 50% Alcohol Solution
C	0.20 gr./100 cc. 50% Alcohol Solution
	Paranitrophenol
A	0.10 gr./100 cc. H_2O
B	0.20 gr./100 cc. H_2O
C	0.40 gr./100 cc. H_2O
	Metanitrophenol
A	0.30 gr./100 cc. H_2O
B	0 60 gr./100 cc. H_2O
C	1.20 gr./100 cc. H_2O

The *p*H of the several indicator solutions was measured electrometrically by means of glass electrode, and the data (Table 2) illustrate that for each of the nitrophenol indicators the *p*H decreases markedly as the concentration increases.

TABLE 2.—*pH of indicator solution*

INDICATOR	A	B	C
Gamma (2:5) dinitrophenol	4.22	4.08*	3.90*
Paranitrophenol	4.95	4.83	4.69
Metanitrophenol	4.90	4.69	4.61

* Indicator dissolved in a 50% by volume alcohol-distilled water solution.

The effect on the buffer solutions of increasing the concentration of indicator was next investigated. Again by electrometric means the *p*H of the buffer solution was first measured. Then to the buffer solution was added indicator corresponding to concentration A, but in the proportion of 1 cc. for each 40 cc. of buffer. The *p*H was again read. This procedure was repeated on systems making use of indicator concentrations B and C, and the relationship of 1 cc. indicator solution to 40 cc. of buffer was maintained. As might be expected, increased concentration of indicator

had relatively little effect on the *p*H of the buffer solution. The accompanying data substantiate this observation (Table 3).

TABLE 3.—*Effect of indicator concentration on pH of buffers*

INDICATOR	BUFFER	NO INDICATOR	A	B	C
Gamma (2:5) dinitrophenol	5.0	5.00	5.04	5 05	5.06
Paranitrophenol	6.0	6.01	6.01	6.01	6 01
Metanitrophenol	7.6	7.57	7.57	7.57	7.55

Finally, by electrometric measurement, *p*H was determined on a variety of cereal and baked products without any indicator present, and then with the proper volume of indicator solution present in concentrations A, B, and C, respectively. In general, there is a decrease in *p*H as the concentration of indicator increases. The magnitude of this change, however, varies with the indicator used and the product tested. The data covering this point are given in Table 4.

TABLE 4.—*Effect of indicator concentration on pH of extracts*

EXTRACT	INDICATOR*	NO INDICATOR	A	B	C
Bread	G.D.N.	5.40	5.35	5.33	5 23
Bread	P.N.	5.58	5.48	5 38	5.38
Cracker crumbs A	P.N.	7.32	7.12	6.96	6.72
Cracker crumbs A	M.N.	7.22	7.22	7.14	7.05
Cracker crumbs B	P.N.	7.46	7 35	7.28	7 15
Cracker crumbs B	M.N.	7.42	7.39	7.32	7 28
Cake	P.N.	7.62	7.28	7 05	6.85
Cake	M.N.	7.58	7.47	7.36	7.20
Flour, bleached	G.D.N.	5.68	5.55	5.48	5.32
Flour, bleached	P.N.	5.63	5.63	5.63	5 63
Flour, unbleached	P.N.	6.24	6.21	6.17	6.10
Macaroni	P.N.	6.28	6.25	6.22	6.17
Whole crackers A	P.N.	7.05	6.80	6 68	6.52
Whole crackers A	M.N.	7.10	7.00	6.92	6.88
Whole crackers B	M.N.	7.58	7.23	7.12	6.99

* G.D.N., Gamma (2:5) dinitrophenol; P.N., Paranitrophenol; and M.N., Metanitrophenol.

From these data (Table 4) it would appear on the surface that the use of indicator concentrations in excess of A would be undesirable from the standpoint of introducing a possible source of error. Nevertheless, colorimetric measurements were made on a variety of products by utilizing the three indicator concentrations, and these results were compared with electrometric measurements. The data given (Table 5) were obtained by electrometrically measuring *p*H and then making the same measurement colorimetrically at the three indicator concentrations. The data presented in Table 6 were obtained to serve as a guard against precon-

ceived ideas influencing the results given in Table 5, by colorimetrically measuring pH at the three indicator concentrations, and making use of unknown extracts. Subsequently these data were checked with electrometric readings.

TABLE 5.—*pH measurements on extracts*

SAMPLE	GLASS ELECTRODE	COLOR. A	COLOR. B	COLOR. C
Bread	5.35	5.40	5.40	5.30
Cake	7.28	7.3	7.3	7.3
Crackers, whole	7.00	6.8	6.8	6.8
Cracker crumbs	7.06	7.0	7.0	6.8
Macaroni	6.26	6.30	6.30	6.30
Flour, unbleached	6.20	6 30	6.30	6.20
Flour, bleached	5 61	5.70	5.70	5.70

TABLE 6.—*pH measurements on extracts (check on Table 5)*

SAMPLE	GLASS ELECTRODE	COLOR. A	COLOR. B	COLOR. C
Bread	5.36	5.4	5.4	5.3
Cake	7.31	7.2	7.1	7.2
Crackers, whole	7.20	7.2	7.1	7.0
Cracker crumbs	7.20	7.2	7.1	7.1
Macaroni	6.27	6.2	6.1	6.3
Flour, unbleached	6.20	6.2	6.0	6.0
Flour, bleached	5.60	5.5	5.4	5.4

Actual application of greater concentrations of indicator to colorimetric measurement in comparison with electrometric measurement fails to substantiate the question raised by the data in Table 4. On the contrary, the data obtained in Tables 5 and 6 in general indicate substantially good agreement between electrometric and colorimetric results regardless of the concentration of indicator used.

It may be concluded, therefore, that in the interest of making color matching easier when working with nitrophenol indicators, a concentration corresponding to C may be used, apparently without affecting the pH result obtained.

STABILIZING FILTERED EXTRACT

In the course of the 1936 work, discrepancies in the electrometric results of two laboratories on crackers gave rise to the belief that a possible source of error existed through failure to limit the time interval between filtration and pH measurement. The probability was expressed that bacterial action takes place with a progressive lengthening of this period, accompanied by a lowering of the pH. It was recommended, therefore, that some effort be made to stabilize the filtered extract.

To accomplish this, boiling distilled water was used in effecting the suspension. This not only was ineffective, but it materially added to the filtration difficulties by gelatinizing the starches. Hydrogen peroxide and formaldehyde were tried, but they can not be used because they lower the *p*H reading. Phenol, acetone, and xylol are ineffective.

Faced with these negative results, the Associate Referee reconsidered this point, and now believes that the most practical manner in which provision can be made against this possibility is to include in the method a statement of caution to measure *p*H as quickly as possible after filtration.

STUDY OF LIGHT CONDITIONS

The conditions under which the samples are compared with the color standards are important. While light intensity is a factor, it is not so essential as a suitable background, such as diffused natural daylight or that furnished by Corning daylight glass (one side ground), or the combination of ground glass and cobalt blue glass.

Investigation induces the belief that an arbitrary definition of light conditions is neither necessary nor desirable. Generally speaking, as the concentration of indicator increases, greater color differentiation is obtained with relatively greater light intensity. Corollary to this observation is the one that when working in the lower (approaching colorless) range of the respective indicators, lesser light intensity is more conducive to color differentiation, while in the upper (yellow colored) range of these indicators greater light intensity favors satisfactory color matching. Purely for the guidance of those who might be interested, it is the opinion of the Associate Referee that for average conditions a light intensity of 15–20 microamperes, as registered through a Weston photronic cell, is adequate.

CONCLUSION AND RECOMMENDATION[1]

As the result of the limited work done this year, it is believed that the findings warrant no addition to Recommendation 6 in the 1936 report, and that in connection therewith (a) concentration C be included in the collaborative investigation, and (b) reaction of collaborators be obtained in regard to the utility of the general specifications referred to herein relative to background and light intensity in carrying out color matching.

The Associate Referee desires to express his thanks and appreciation to W. Reiman, who not only did the work reported herein, but assisted in the preparation of this report.

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 75 (1938).

REPORT ON STARCH IN FLOUR

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington D. C.), *Associate Referee*

The collaborative work on starch has been almost continuous since 1927, principally on the Rask method and its modifications. Until this year, the collaborative work was done on flours, and fairly good results were reported. However, it is a method requiring experience on the part of the analyst to obtain reliable results. It is apparently specific for starch and relatively rapid, but seems limited in application to finely ground cereal flours.

Before sending out the collaborative samples this year, the Associate Referee had applied the method to bread on several occasions. Some samples of bread gave acceptable results, but an occasional sample gave unreliable results.

In order to determine by collaborative work the application of the method to macaroni and cooked products, as bread and cake, samples were sent to six collaborators with the request that starch be determined by the modified Rask method, *Methods of Analysis, A.O.A.C.*, 1935, page 213. The results on the cake and macaroni are shown in the table. The bread was not analyzed by the collaborators, due to later instruction that the method was not applicable to the particular sample submitted.

The results are shown in the table.

The results on the macaroni are fair; those on the cake are unsatisfactory. Likewise the results on the one sample of bread analyzed are unsatisfactory. On the basis of these collaborative results and the experience of the Associate Referee, it may be concluded that this method is not satisfactory for cooked products.

Several of the methods published in recent years on the determination of starch are based on extraction with hot calcium chloride solution, and various modifications are given for the actual measurement. The modified Mannich-Lenz method studied last year on flour, *Can. J. Research*, **11**, 751 (1934), the method published in *This Journal*, **18**, 621 (1935); the method published in *Ind. Eng. Chem. Anal. Ed.*, **8**, 92 (1936); and the method published in *Mitt. Lebensm. Hyg.*, **28**, 111 (1937) are cited for reference and possible application. It seems probable that one of these methods, or modifications of the same, may be satisfactorily applied, not only to flour and macaroni but also to noodles and all bakery products. It is also probable that a uniform method could be developed to replace the numerous methods now given under the various classes of foods in *Methods of Analysis, A.O.A.C.* for the determination of starch.

The Association appreciates the cooperation of the following collaborators in this work:

C. H. Robinson, Central Expt. Farm, Dept. of Agriculture, Ottawa, Canada.

W. F. Geddes, Grain Research Lab., Dept. of Trade and Commerce, Winnipeg, Canada.
C. Y. Hopkins, Division of Chemistry, National Research Council, Ottawa, Canada.
Stephen Voris, Loose-Wiles Biscuit Co., Long Island City, N. Y.
Sidney Goren, Dept. of Biochemistry, The Johns Hopkins University, Baltimore, Md.

Results on cake and macaroni samples by the Rask method

COLLABORATOR	CAKE	MACARONI
	per cent	*per cent*
1	17.52	67.48
	20.00	66.66
	17.62	67.86
	18.72	
	21.60	Av. 67.33
	20.56	
	17.54	
	20.00	
	Av. 19.2	
2	14.95	70.32
	13.85	69.53
	14.39	69.22
	Av. 14 39	Av. 69.69
3	20.20	67.60
	20.80	67.40
	Av. 20.50	Av. 67.50
4	26.20	67.28
	28.15	70.72
	20.27	65 70
	23.27	68.88
	25.77	70.18
	23.31	70.44
	25.85	72.86
	Av. 24.69	Av. 69.44
5	23.20	69 80
	23.08	68.96
		68.48
	Av. 23.1	
		Av. 69.1
6	15–21%	62.10
Note: Collaborator 5 obtained on the bread:		65.79
per cent		63.69
42.96 31.96		64.09
44.80 36.28		Av. 63.9
34.16		

RECOMMENDATIONS[1]

It is recommended—

(1) That no more collaborative work be done at this time on the modified Rask method.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 75 (1938).

(2) That the modified Rask method be limited in its application to cereal flours.

(3) That further study on the determination of starch be confined to the development of a method applicable to all cereal products, cooked and uncooked.

REPORT ON FLOUR-BLEACHING CHEMICALS*

By DOROTHY B. SCOTT (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

The recommendation of Subcommittee D that the method by Munsey for the determination of chlorine bleach be studied collaboratively was partly carried out by submitting to six collaborators three samples of flour bleached with known amounts of Agene.

The bleaching of flour by the Agene process (nitrogen trichloride gas) adds chlorine to the fat of the flour in very small amounts.

The samples sent to the collaborators contained no Agene, and the minimum and maximum amounts used generally in commercial practice, respectively.

Three collaborators, all members of the Food and Drug Administration, submitted results as follows:

COLLABORATORS	SAMPLE A—NO AGENE	SAMPLE B—1 GRAM OF AGENE PER BBL.	SAMPLE C—5 GRAMS OF AGENE PER BBL.
	chlorine (p.p.m.)	*chlorine (p.p.m.)*	*chlorine (p.p.m.)*
J. S. Ard, New York	1.3	1.9	4.7
W. E. Kirby, New York	0.83	2.02	4.04
V. E. Munsey, Washington	0.76, 1.0 Av. 0.88	1.67, 1.83 Av. 1.75	3.77, 3.74 Av. 3.76
Average	0.97	1.86	4.06

The agreement on Sample B is good.

COMMENTS ON THE METHOD

J. S. Ard.—The instructions should say to do the blank not on 700 cc. of gasoline as called for in the method, but on the same amount as the flour filtrate, or the average flour filtrate of 475 cc. If 700 cc. is run through the filter paper, then all but 475 cc. should be discarded.

When the charred fat was ignited the samples behaved all right, but it was noticed that the blank had not become converted to carbonate but fused easily to very active alkali, which appeared to be a great danger to the platinum dish. The blank was therefore kept fused just long enough to dissolve the slight char present (about 4 minutes), and it was not put in the muffle with the samples.

W. E. Kirby.—Sample C is probably too high since the first drop of KCNS gave the end point.

* Presented by H. K. Parker.

DISCUSSION

The consensus of opinion of the analysts familiar with the method has been that Ard's comment on the advisability of using a constant of 475 cc. of gasoline in the blank would increase the accuracy of the method.

The bad effect of the alkali on the platinum dish as commented on by Ard was disproved by Hogan of the New York Station, who found a very small loss of weight (about 0.006 gram) in the platinum dish used in the blank determination.

Experience at the New York Station in analyzing commercial samples has shown that it is often necessary to add more than the 5 cc. of the silver nitrate solution, as suggested by Kirby. As much as 10 cc. has been added, and it is recommended that this amount be stated in the method.

Since the bleaching of flour by chlorine is determined by the quantity in the extracted fat rather than in the whole flour, it is suggested that the results be reported in mg. per gram of fat. This would eliminate the necessity of making moisture determinations, the variables caused by the indefinite quantities of gasoline recovered, and the evaporation of gasoline during filtration, and would shorten the method. Before making this modification, however, it would be necessary to determine whether any chlorine is lost when the fat is dried and weighed before the alkali is added.

The study of the method for the detection of benzoyl peroxide, with the purpose of making it quantitative, was continued but not enough progress was made for a report. The Associate Referee intends to continue this study.

Munsey has worked out a new method to replace the gasoline color value. It is based on the measurement of total carotinoid pigments in bleached and unbleached flour with the neutral wedge photometer. This method should be studied collaboratively.

RECOMMENDATIONS[1]

It is recommended—

(1) That the modifications of Munsey's method for the determination of small amounts of chlorine be studied, and the method again be submitted to collaborative study.

(2) That the method for the detection of benzoyl peroxide bleach in flour be further studied.

(3) That Munsey's method for the measurement of total carotinoid pigments in bleached and unbleached flour with the neutral wedge photometer be submitted for collaborative study.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 21, 75 (1938).

REPORT ON CARBON DIOXIDE IN SELF-RISING FLOUR

By HOWARD ADLER* (Victor Chemical Works, Chicago, Ill.), *Associate Referee*

Last year's report, *This Journal*, **20**, 365–369 (1937), on the determination of carbon dioxide in self-rising flour suggested that an attempt would be made to determine what variables influence the carbon dioxide recovery. The work was undertaken by R. A. Barackman in the laboratory of the Referee as this was considered a better plan than that of testing of samples of self-rising flour by a larger number of collaborators.

Previously published work on this problem includes that of Hertwig and Hicks in 1928,[1] who called attention to a discrepancy due to vapor pressure of the acid solution in gasometric determinations of carbon dioxide. They noted a greater displacement in the gas measuring tube than the volume of added acid by amounts of 3–5 cc. Chittick, Dunlap, and Richards in 1930,[2] working with baking powders, concluded that "whatever vapor tension effect is produced by the liquid in the reaction flask is closely compensated for by the carbon dioxide dissolved in the liquid." These workers made no reference to a like study on self-rising flours.

Whiting in 1932, *This Journal*, **15**, 588, noted that the maximum and minimum results between collaborators were too widely divergent and suggested that the cause might be due to a loss of carbon dioxide by chemical action or a retention of some gas by the flour-acid mixture, the latter being the more likely. He suggested that further work be done to determine whether this amount is constant for all flours and for all percentages of carbon dioxide. Whiting showed less carbon dioxide recovery from self-rising flours containing higher percentages of sodium bicarbonate.

The present Associate Referee attempted to determine such variables as cause a serious error in the method, but keeping in mind the practical limits for a rapid method. H. V. Moss, in a private communication to the Associate Referee, stated that if 8.5 grams of self-rising flour is used instead of 17, with 25 cc. of 1+5 acid instead of 50, the recovery is increased, due to the decrease in the amount of solution, which probably acts as a solvent for carbon dioxide, because when 8.5 grams of flour with 50 cc. acid is run, the lower recovery is also obtained. He also showed that with water in place of 1+5 acid, there is still lower recovery.

EXPERIMENTAL

Method A, *This Journal*, **20**, 365 (1937); **15**, 588 (1932), modified to use 17 grams of self-rising flour, was used throughout this work except where noted. The data presented are simplified to give only the percentage

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* Presented by R. A. Barackman.
[1] *Cereal Chem.*, **5**, 482–4.
[2] *Ibid.*, **7**, 473.

of carbon dioxide obtained since no correlation was observed between recovered carbon dioxide and the date of the tests, the temperature, or the atmospheric pressure. Self-rising flours were carefully blended each day that tests were run, and 1.5 per cent sodium bicarbonate, 1.875 per cent calcium acid phosphate, and 1.75 per cent sodium chloride to 100 parts of flour were used. The theoretical amount of carbon dioxide that should be recovered from these flours was 0.7475 per cent.

The four soft wheat flours used had the following analyses:

FLOUR	MOISTURE	ASH
	per cent	*per cent*
A—Standard Patent	11.7	0.40
B—Extra Short Patent	12.8	0.30
C—Short Patent	12.5	0.35
D—Cut Straight	12.5	0.46

The effect of varying quantities of reactants, acid volume, and acid concentration is shown in Tables 1, 2, and 3.

TABLE 1.—*Effect of reduced reactants*

S.R.F. (grams)	17	12.5	8 5	8.5
Acid (1+5) (cc.)	40	30	20	40
FLOUR	CO_2 IN S.R.F. (PER CENT)			
A	.720	.736	.788	.748
B	.724	.736	.755	.728
C	.715	.750	.782	.762
D	.694	.738	744	.740
A	.677	.735	.810	—
A	.718	.757	.790	.752
A	.738	.778	.819	.790
Average	.712	.747	.784	.752

TABLE 2.—*Effect of variation of acid volume—17 grams S.R.F. constant*

Acid (1+5) (cc.)	50	45	40	35	30	25
FLOUR	CO_2 FROM S.R.F. (PER CENT)					
A	—	.695	.710	.724	.726	(.712)v
B	.703	.710	.724	.748	.758	(.750)v
C	.692	.697	.707	.719	.729	.739
D	.690	.688	.703	.703	.724	.745
Average	.695	.698	.711	.724	.734	.742

v = viscous suspensions; very difficult to obtain a full release of CO_2; not used in calculation of average.

The results show a wide variation in carbon dioxide recovery from freshly prepared self-rising flours. Table 4, discussed later, indicates the extent of variation expected when five replica determinations are made on the same sample. All figures in Tables 1, 2, and 3 are from single determinations, but the averages by any one method are comparable and may be discussed. It will be noted that a reduction to one-half the amount of self-rising flour and acid causes a recovery of carbon dioxide greater than theoretical. This will be explained later. The use of three-fourths of the specified reactants gave results very close to theoretical. The use of one-half the amount of self-rising flour but with the full volume of acid also gave greater than theoretical recovery.

Table 2 shows an increasing recovery of carbon dioxide, with decreasing amounts of added acid. The use of 17 grams of self-rising flour plus 30 cc. of 1+5 sulfuric acid gave the greatest recovery without the unfavorable condition of insufficient liquid to make a suspension capable of releasing carbon dioxide.

Table 3 shows a constant recovery of carbon dioxide with variable acid strength. Solution of carbon dioxide is most likely the cause of recovery lower than theoretical.

TABLE 3.—*Effect of variation of acid concentration—17 grams S.R.F. and 45 cc. acid constants*

Acid Dilution	(1+5)	(1+6)	(1+8)	(1+10)	(1+12)
FLOUR	CO_2 FROM S.R.F. (PER CENT)				
A	.712	.709	.715	.709	.709
B	.711	.708	.709	.709	.715
C	.706	.702	.698	.678	.696
D	.711	.705	.715	.705	.701
Average	.710	.706	.709	.700	.705

A second cause for error in carbon dioxide determinations is that suggested by Hertwig and Hicks. This cause, and the fact that more than theoretical values were obtained previously, led to tests in which corrections of gas volume were made after the volume displacement of the acid as well as that due to plain flour plus acid had been determined. An example of the results of replica determinations is given in Table 4.

An error of from 3.5 to 5.5 cc. excess displacement occurred when Modified Method A was used (Table 4). This is in agreement with the findings of Hertwig and Hicks. An effort was made to find at what acid-S.R.F. ratio this error might become negligible. It should be noted here that 250 cc. reaction flasks were used in all tests reported in this paper. With increasing volume of acid or of acid plus plain flour, the amount of

TABLE 4.—*Replica determinations of carbon dioxide—corrections applied for displacement volume of acid and acid plus plain flour—17 grams flour or S.R.F.*

ACID ADDED	VOLUME DISPLACEMENT		CO_2	CO_2 AFTER CORRECTING FOR VOLUME DISPLACED BY—	
	ACID	ACID+PLAIN FLOUR		ACID	ACID+FLOUR
grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
40	43.5	43 5	.711	.678	.678
40	44.5	44.0	.706	.661	.666
40	44.5	44.0	.706	.661	.666
40	44.0	44.5	.719	680	.675
40	45.5	45.5	.712	.659	.659
Average	44.25	44.12	.711	.668	.669

displacement became less (Table 5), but also the amount of recovered carbon dioxide became less, off-setting any advantages in the use of a larger volume of acid.

TABLE 5.—*Effect on gas volume of acid and acid plus plain flour* (acid used = 1 + 5 H_2SO_4)

WT. PLAIN FLOUR OR S.R.F.	NO. OF DETNS.	LIQUID	VOLUME OF LIQUID	EXCESS VOLUME DUE TO—		CO_2	CO_2 AFTER CORRECTING FOR VOLUME DISPLACED BY—		FLASK T CORRECTION
				LIQUID	TO LIQUID PLUS PLAIN FLOUR		ACID	ACID+PLAIN FLOUR	
grams			*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
17.0	2	Acid	25	4.0	4 0	.727	.685	.689	007
17.0	3	Acid	30	4 8	4 0	723	.674	.682	008
17.0	5	Acid	40	4.25	4.12	711	.668	669	008
17.0	2	Acid	60	3.75	2.75	.681	643	.653	005
17.0	2	Acid	80	—	3.0	—	—	—	—
17.0	2	Acid	100	—	4 0	—	—	—	—
17.0	2	Acid	150	—	1.25	—	—	—	—
17 0	2	Acid	200	—	1.0	—	—	—	—
8.5	5	Acid	20	5.40	4 25	782	.676	700	005
17.0	2	Water	40	7.75	4.50	.498	422	454	005
8.5	2	Water	20	5.75	5 75	.678	.563	563	.005
17.0	2	Water	30	6.00	5.75	.574	.516	.517	.004

Corrections for volume displacement of acid and acid plus plain flour when applied in the calculation of percentage of carbon dioxide in self-rising flour gave results lower than when the acid volume only was used. The use of half quantities of S.R.F. and acid showed the greatest error to occur with the published method.

When water was substituted for the 1+5 acid mixture, consistently less carbon dioxide was recovered in comparison with results from similar acid-S.R.F. ratios. These data substantiate the fact noted above that carbon dioxide remains dissolved in the water in the reaction flask.

In all the work reported here, room temperature was used for factoring temperature and pressure effects on gas volume. The temperature of the flask contents was also obtained for all determinations and was from 1 to 2° C. higher than room temperature. Calculations in which the higher temperature was used resulted in percentage carbon dioxide values lower by the amounts shown in the last column of Table 5. This temperature differential may be due to mechanical agitation of the flask, but is more likely due to heat of solution of self-rising flour as well as leavening ingredients. It will be noted that the values are low with water, and higher with 1+5 acid. The writer has experienced as high as 10° C. temperature differences when using 1+1 in place of 1+5 acid. It is possible that flour-acid reaction is also a source of heat. However, the influence of 2° difference in temperature is relatively constant so that calculations involving the use of room temperature are reliable.

TABLE 6.—*Effect of self-rising flour ingredients on carbon dioxide recovery*
(17 grams flour mix+40 cc. 1+5 acid, correction applied for volume displacement of acid plus plain flour, and method described above used)

MIX NO.	COMPOSITION OF MIXES				CO_2*
	FLOUR	SODA	PHOSPHATE	SALT	
	grams	*grams*	*grams*	*grams*	*per cent*
1	100.0	1.5	1.875	1.75	.692
2	101.75	1.5	1.875	—	.682
3	101.875	1.5	—	1.75	.672
4	100 (starch)	1.5	1.875	1.75	.731
1	100	1.5	1.875	1.75	.688
4	100 (starch)	1.5	1.875	1.75	.722

* Duplicate determinations.

Vapor tension of the acid-S.R.F. suspension appears to be a source of large error. The tests recorded previously were run during the summer months when the air was warm and relatively dry. Later tests with 40 cc. of acid with 17 grams of plain flour showed a displacement volume of 40 cc. when the temperature of the room was normal but when the air was relatively saturated with water vapor. This indicated that when a 250 cc. reaction flask is used with approximately 200 cc. of air space over the liquid, the source of error due to vapor tension may vary with the relative humidity. Unfortunately, no records were kept this year to establish this point, but it may be corrected for by use of the acid or plain

flour plus acid volume displacement rather than the volume of added acid.

The effect of the four ingredients of self-rising flour was determined as shown in Table 6.

It appears that in the past low recovery of carbon dioxide has been attributed to solution of gas by the liquid. Table 6 shows that flour plays a large part in retaining carbon dioxide. It may be possible to explain discrepancies in past collaborative work from the standpoint of soda-flour or CO_2-flour reactions. That such reactions occur is evidenced from the work of Whiting, where lower recovery was obtained from self-rising flours containing increasing amounts of leavening. This effect of flour has not been overcome.

DISCUSSION

The gasometric method of determining carbon dioxide in self-rising flour is subject to errors that result in recovery lower than theoretical. In an attempt to eliminate the loss, the Associate Referee studied the effects of varying the quantities of reactants, and the volume of acid used, as well as the concentration of the acid solution. The influence of volume displacement of acid added to the dry decomposition flask and to plain flour was also studied, and this factor was used as a correction in the calculations of recovered carbon dioxide.

No single factor was found to be accountable for the loss of carbon dioxide, one seeming to offset another to some extent. Significant errors are due to volume displacement in excess of volume of acid added, to solution of carbon dioxide, and to retention of carbon dioxide by the self-rising flour-acid suspension. It does not appear likely that any simple way of eliminating the effects of these factors so as to get complete recovery of the gas evolved will be found.

The official gasometric method, *Methods of Analysis, A.O.A.C.*, 1935, 208, will give results satisfactory for all practical purposes. Whether the same factor for the conversion of carbon dioxide recovered to carbon dioxide present can be used in laboratories in different locations remains to be determined.

REPORT ON MILK SOLIDS IN MILK BREAD

By V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The so-called fat method for the determination of milk solids, published in *Methods of Analysis, A.O.A.C.*, 1935, page 222, has been subjected to collaborative study for the past two years. The results have been somewhat disappointing. Accordingly, the work this year was confined to a study of some of the factors that may be the cause of the variation in

collaborative results. The method is apparently satisfactory up to the determination of the so-called fat number, which is essentially the Reichert-Meissl procedure, with modifications in the weight of sample and strength of titrating reagent. The fat number procedure was studied this year on three samples of uniformly mixed fats of hydrogenated oil and butterfat, corresponding in composition to the fat extracted from a water bread, a half-milk bread, and a milk bread, respectively. These samples were sent to six collaborators, who had previously worked on bread samples, with the request that they determine the fat number. The results are given in Table 1.

TABLE 1.—*Fat numbers*

COLLABORATOR	SAMPLE 1	SAMPLE 2	SAMPLE 3
1	0.90	7.45	16.21
	1.01	7.27	16.39
	Av. 1.0	Av. 7.4	Av. 16.3
2	1.93	9.80	17.40
	1.77	9.63	17.11
	Av. 1.9	Av. 9.7	Av. 17.3
3	3.9	11.6	18.3
	3.4	11.2	18.5
	Av. 3.7	Av. 11.4	Av. 18.4
4	1.65	9.32	17.30
	1.62	9.38	17.10
			17.15
	Av. 1.6	Av. 9.4	Av. 17.2
5	1.92	9.75	17.38
	1.76	9.59	17.89
	Av. 1.8	Av. 9.7	Av. 17.6
6	1.1	8.3	15.7
7	1.33	8.2	15.4
	1.30	9.4	16.6
	Av. 1.32	Av. 8.8	Av. 16.0
	Max. 3.7	11.4	18.4
	Min. 1.0	7.4	15.7
	Av. 1.8	9.3	16.9

These results (Table 1) are similar to those obtained on bread for the past few years. They indicate that the variation obtained on bread is due to the distillation procedure for determining the fat number, and not to the procedure for extraction of the fat.

The sodium hydroxide saponification was utilized in this work to avoid the large blank resulting from the glycerol and sodium hydroxide mixture used in the regular Reichert-Meissl procedure. The sodium hydroxide blank was considered to be insignificant for the results obtained by the Associate Referee. Accordingly, no emphasis was placed on the necessity of using recently boiled distilled water and clear sodium hydroxide solu-

tion free from carbonates. It is possible that there may be some variation in the amount of carbon dioxide in the water and the carbonates in the sodium hydroxide used by the different analysts. To avoid this effect and any unsuspected irregularities, it is recommended that a blank determination be made on any later determinations.

The use of a 1 gram sample of fat and 0.01 *N* sodium hydroxide in this empirical fat number procedure may possibly magnify the inherent errors more than in the case of the regular Reichert-Meissl procedure. In order to study this point collaboratively three similar fat samples, but not the same mixture, were submitted to the same collaborators, with the request that the Reichert-Meissl number be determined by the regular official method. The results obtained are shown in Table 2.

TABLE 2.—*Reichert-Meissl numbers*

COLLABORATOR	SAMPLE 1	SAMPLE 2	SAMPLE 3
1			
2	0 70	6.97	13 22
	0 75	6 94	13 33
	Av. 0.73	Av. 6.96	Av. 13.28
3	0 7	5 6	10 5
	0.7	5 8	10 5
	Av. 0.7	Av. 5.7	Av. 10 5
4	0.76	6 54	12 88
	0.67	6.68	12 84
	Av. 0.72	Av. 6 61	Av. 12.86
5	—	—	—
6	0.61	5.78	11 28
7	1.18	7 03	13 21
	1.13	7.08	13.23
	Av. 1.15	Av. 7 06	Av. 13 22
	Max. 1.2	7 1	13 3
	Min. 0.6	5.7	10 5

Since the results on the Reichert-Meissl numbers do not show a marked improvement over those obtained by the fat number procedure, it does not seem desirable to recommend that the regular Reichert-Meissl procedure replace the fat number procedure. It would require the extraction of four or more bread samples to get sufficient fat, instead of the present extraction of two samples. The fat method was not applied to bread, as such, this year, and since it is convenient to study all these methods on the same samples, neither were the citric acid and lactose methods studied.

This report is made possible by the generous cooperation of the following collaborators, to whom many thanks are due:

E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.
J. H. Bornmann, U. S. Food and Drug Administration, Chicago, Ill.

Fred Hillig, U. S. Food and Drug Administration, Washington, D. C.
Charles Hoffman, Ward Baking Company, New York, N. Y.
W. Catesby Jones, Division of Chemistry, Dept. of Agriculture and Immigration, Richmond, Va.
R. S. McKinney, Bureau of Chemistry and Soils, Washington, D. C.

RECOMMENDATIONS[1]

It is recommended—

(1) That the citric acid method be further studied collaboratively.

(2) That the fat method be modified to include a blank determination, and that collaborative work be continued under more detailed directions.

(3) That a study of the lactose method be continued, with a view to collaborative study should the facts warrant.

No report on viscosity of flour was given by the associate referee.

REPORT ON COLD WATER EXTRACT IN FLOUR

By H. C. Fellows (Bureau of Agricultural Economics, Washington, D. C.), *Associate Referee*

This year a study was made of the efficiency of the tentative method for the determination of cold water extract, *Methods of Analysis, A.O.A.C.*, 1935, 213. For this purpose three flours were chosen: A soft wheat patent flour, a soft wheat straight grade flour, and a soft wheat first clear flour. Samples of these flours were sent out to a number of collaborators.

The collaborative results are given in the table.

Per cent cold water extract

COLLABORATOR	PATENT FLOUR	STRAIGHT FLOUR	FIRST CLEAR FLOUR
1	7.59	7.56	8.46
2	4.73	4.84	5.28
3	3.86	3.71	4.15
4	3.61	3.53	4.14
5	5.02	4.52	5.29
6	4.00	3.96	4.46
7	5.47	5.42	6.15
8*	4.64	4.56	5.51
8A†	4.08	3.90	4.27
9	3.96	3.67	4.36
10	4.22	3.94	4.33
11	3.93	3.92	4.35
12	3.76	3.58	4.04

* Run at 46° F. in a Frigidaire.
† Run in an ice bath at 32° F.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 76 (1938).

The results show considerable variation among the collaborators. Comments were made by several of the collaborators, especially in regard to clearness of filtration. The patent flour gave a turbid extract. Centrifuging was suggested as a help, but it is not mentioned in the procedure. This condition brings up the question of suitable filter paper.

The results submitted by collaborators 8 and 8A (46° F. extraction versus 32° F. extraction), indicate that temperature is an important factor. The colder temperature will hold in check to a greater degree any enzymatic activity that may affect the results of the determination.

Another important factor is the proper drying temperature for the extract. The Associate Referee has experimented with this factor in determining the percentage of total solids in cold water extract (32° F.) in barley malt, especially from the standpoint of the caramelization of the sugars. It was found that 70° C. under vacuum gave good results.

The collaborators were as follows:

1. Howard M. Simmons, Mid-West Laboratories Co., Inc., Columbus, Ohio.
2. William H. Cathcart, American Institute of Baking, Chicago, Ill.
3. L. W. Haas, The W. E. Long Co., Chicago, Ill.
4. M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.
5. Rae H. Harris, Agricultural Experiment Station, Fargo, N. D.
6. Lawrence Zeleny, Bureau of Agricultural Economics, U. S. Dept. Agriculture, Washington, D. C.
7. M. H. Neustadt, Bureau of Agricultural Engineering, U. S. Dept. Agriculture, Washington, D. C.

8 and 8A. H. C. Fellows.

9. C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.
10. L. D. Whiting, Ballard and Ballard, Louisville, Ky.
11. F. A. Collatz, General Mills, Inc., Minneapolis, Minn.
12. H. W. Putnam, Igleheart Bros. Inc., Evansville, Ind.

It is recommended[1] that study of the method be continued, and that special consideration be given to the following points: Grade of filter paper, time of extraction, extraction temperature, and drying temperature.

No report on ergot was given by the associate referee.

REPORT ON CATALASE AND PROTEOLYTIC ENZYMES

By W. S. Hale (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

It is recommended[2]—

(1) That the work on catalase in flours be dropped until further interest or need is shown for the continuation of this work.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 76 (1938).
[2] For report of Subcommittee D and action by the Association, see *This Journal*, 21, 75 (1938)

(2) That work on proteolytic enzymes of flour be continued, as there is considerable interest in this subject and a definite program for the continuation of this work for next year is indicated.

Mr. Hale also discussed informally the subject of lipase in flour.

No formal report on color in flour was given by the associate referee.

No report on soya flour in foods was given by the associate referee.

REPORT ON MACARONI

By W. F. GEDDES (Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Canada), *Associate Referee*

The object of the present study is the development of a method for identifying the farinaceous ingredient (or ingredients) present in macaroni.

This problem is being attacked from a synthetic angle. Semolina and flour of 50, 60, and 70 per cent extraction were experimentally milled from a single sample of durum wheat; farina and flour of 50, 60, and 70 per cent extraction were milled from a sample of hard red spring wheat; and macaroni was processed from all the milled products.

Various chemical determinations were made upon the wheats, mill products, and the macaroni, but little information of value has so far been secured. The protein contents of the flours and the macaroni processed therefrom are decidedly greater than the corresponding values for semolina and farina. A similar relationship, which extended through into the finished product, was found between the durum flours and semolina carotenes when determined by extraction with water-saturated n-butyl alcohol. The macaroni prepared from hard red spring wheat, however, showed little if any variation in pigment content irrespective of whether farina or flour was used in its production. The ether extract appeared to increase when flour was used, but no distinction was found between hard red spring and durum wheat.

The ash content of the macaroni did not appear to be a very valuable index; while a slight increase was found when flour was used instead of farina, no such relationship appeared to exist between the flour and semolina. There was some indication that the equilibrium moisture content of the finished macaroni is greater when flour of low extraction is used, and as the normal equilibrium moisture content of durum macaroni is quite constant irrespective of variety or processing treatment, an investigation of this point may be of some value and will be looked into

shortly. The water absorption of the dough was definitely greater when flour was used, which is probably associated with the tendency towards higher equilibrium values.

The color of the macaronis produced from the various flours was much deeper than when either semolina or farina was used. The transverse breaking strength of the macaroni increased markedly from semolina or farina to flour of 50 per cent extraction, with a tendency to decrease as the per cent extraction increased.

Special apparatus is being developed for an investigation of the cooking characteristics and for the measurement of physical properties of the cooked products. It is believed that this line of attack offers the best promise for successfully identifying the farinaceous ingredients of macaroni.

No report on whole wheat flour was given by the associate referee.

No report on phosphated flour was given by the associate referee.

TUESDAY—AFTERNOON SESSION

REPORT ON STANDARD SOLUTIONS

By R. L. Vandaveer (U. S. Food and Drug Administration, Chicago, Ill.), *Referee*

In 1935 the Referee offered a method for the preparation and standardization of sodium hydroxide and hydrochloric acid solutions, *This Journal*, **19**, 46, 194 (1937). These procedures were adopted as tentative by the Association, *Methods of Analysis, A.O.A.C.*, 1935, 681. Last year a procedure formulated by the Referee for standardizing sulfuric acid solutions by weighing ammonium sulfate was found to be somewhat unsatisfactory as a precise measure of titratable acidity, *Ibid.*, **20**, 387 (1937).

Subcommittee A then recommended that collaborative work be done on the methods for sodium hydroxide and hydrochloric acid solutions. The work reported here was performed on 0.1 *N* solutions, because this strength probably is used more frequently than any other concentration, and the results on 0.1 *N* should be applicable to stronger or weaker solutions than 0.1 *N*.

PREPARATION OF STANDARD SODIUM HYDROXIDE AND HYDROCHLORIC ACID

Table 1 records collaborative effort in preparing solutions of exactly 0.1 *N* of both acid and alkali according to the directions outlined in *Methods of Analysis*. In each case the collaborator prepared one liter of solution, standardized it, and finally diluted to 0.1 *N*.

Table 1.—*Preparation of 0.1 N solution*

Collaborator	NaOH normality		HCl normality	
	Original unadjusted strength	After dilution to make 0.1 *N*	Original unadjusted strength	After dilution to make 0.1 *N*
E. H. Berry, Chicago	0.1004	0.1001	0.1041	0.1001
H. R. Bond, Chicago	0.1010	0.1000	0.1038	0.0994
R. L. Vandaveer, Chicago	0.1008	0.1000	0.1025	0.1001

STANDARDIZATION

In the preparation of standard alkali care was taken to insure freedom from carbon dioxide. The solutions were prepared and transported in glass-stoppered Pyrex ware. Table 2 lists the results of collaborators on the submitted solutions of sodium hydroxide and hydrochloric acid according to the tentative procedures.

TABLE 2.—*Collaborative results on standard solutions of NaOH and HCl*

ANALYST	NaOH SOLUTION			HCl SOLUTION		
	ACID POTASSIUM PHTHALATE	TITRATION	NORMALITY	HCl	TITRATION NaOH	NORMALITY
	gram	*ml.*		*ml.*	*ml.*	
J. P. Aumer	0.8189	40.00	0.1003	40.00	39.83	0.0998
New Orleans	0.8179	39.98	0.1002	40.00	39.83	0.0998
	0.8182	40.01	0 1002	40.00	39.83	0.0998
Av.			0.1002			0 0998
E. H. Berry	0.9009	44.02	0.1003	40 00	39.90	0.1001
Chicago	0.9001	43.99	0.1003	40 00	39.85	0.0999
	0.9022	44.05	0.1003	50.00	49.80	0.0999
Av.			0.1003			0.1000
J. Carol	0.8848	43.20	0.1003	39.81	39.70	0.1001
Cincinnati	0.8526	41.59	0.1004	40.03	39.90	0.1001
	0.8749	42.67	0 1004	40.00	39.85	0.1000
Av.			0.1004			0.1001
G. M. Johnson	0.8091	39.60	0.1001	40 00	39 78	0.0996
Minneapolis	0.8296	40.61	0.1001	40.00	39.80	0.0996
	0 7845	38.40	0.1001	40.00	39.81	0.0996
				40.00	39.80	0 0996
				40 00	39.82	0.0996
Av.			0.1001			0.0996
L. Jones	—	—	0.1002	40.00	39.88	0 0999
Kansas City				40.00	39.90	0.1000
			0.1003	50.00	49.78	0.0998
				50.00	49.80	0.0998
			0.1002	50.00	49.80	0.0998
Av.			0.1002			0.0999
A. L. Prince	0.8111	39.70	0.1001	40.00	39.92	0.0998
New Brunswick	0.8064	39.50	0.1000	40.00	39.98	0.1000
	0.8217	40.32	0.0998	40.00	40.00	0.1000
	0.8003	39.25	0.0999			
Av.			0.1000			0 0999
R. L. Vandaveer	0.9955	48.62	0.1003	40.00	39.88	0.0999
	0.9971	48.71	0.1003	50 00	49.81	0.0999
	0.9968	48.70	0.1003	50.00	49 82	0.0999
Av.			0.1003			0.0999

DISCUSSION

The results of the three collaborators in the preparation of standard solutions are quite satisfactory (Table 1). The procedure is simple, and the accuracy is dependent upon the volume measurements.

In the standardization of sodium hydroxide solution the accuracy of the potassium acid phthalate procedure has been established by the National Bureau of Standards[1] (Standard Sample No. 84) and by Hendrixson.[2] The latter investigator also determined that the standardization of acid through alkali against acid potassium phthalate was extremely accurate.

The range in results for the standardization of the alkali is four parts in a thousand. The normalities of the hydrochloric solution vary from 0.0996 to 0.1001. The Referee considers that the collaborative results on both the alkali and acid solutions are in good agreement and indicate that the methods are precise for standardization purposes.

RECOMMENDATIONS[3]

It is recommended—

(1) That other direct methods of standardizing acids be studied.

(2) That the standardization of iodine solution be studied.

(3) That the standardization of sodium thiosulfate solution be studied.

REPORT ON INSECTICIDES, FUNGICIDES AND CAUSTIC POISONS

By J. J. T. Graham (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

During 1937 the Referee gave his attention to methods for the analysis of pyrethrum products and derris and cube powder.

The study of methods for the determination of fluorine compounds, and of naphthalene in poultry lice products was assigned to associate referees. No report will be given by the Associate Referee on Fluorine.

No report on fluorine compounds was given by the associate referee.

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[1] Bureau of Standards Certificate of Analysis.
[2] *J. Am. Chem. Soc.*, 37, 2352 (15).
[3] For report of Subcommittee A and action of the Association, see *This Journal*, 21, 59 (1938).

REPORT ON PYRETHRINS, DERRIS, AND CUBE

By J. J. T. Graham (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year the Referee called attention to the method proposed by Wilcoxon[1] for the determination of Pyrethrin I in pyrethrum powder and recommended that it be studied this year. In view of the fact that D. A. Holaday of the Insecticide Division of the Food and Drug Administration was making an investigation of this method and its application to mineral oil-pyrethrum extracts, no general collaborative assistance was sought.

The Wilcoxon method was found by Holaday to be satisfactory for the determination of Pyrethrin I in pyrethrum powder, but unsatisfactory for this determination in pyrethrum powder mixtures and in mineral oil-pyrethrum extracts. Modifications introduced into the procedure by him,[2] however, make the method applicable for the determination of Pyrethrin I in these products in the presence of essential oils, perfumes, derris resins, and other materials.

The modified method was published in *This Journal*, **21**, 78 (1938).

TABLE 1.*—*Pyrethrin I in alcoholic pyrethrum extracts*

MATERIAL ANALYZED	MERCURY REDUCTION METHOD	SEIL METHOD	MERCURY REDUCTION ON THE TITRATED SOLUTION FROM THE SEIL METHOD
	per cent	*per cent*	*per cent*
Alcoholic pyrethrum extract	0.31	0.27	0 21
	0 32	0.27	0 20
Alcoholic extract+pine oil 5%, oleic acid 4%, and derris resins 2%	0 35	—	—
	0.32	—	—

* Tables 1 and 2 are taken from the paper by D. A. Holaday, previously mentioned

The same quantity of the alcoholic pyrethrum extract was used for each determination. The results in Table 4 show that the addition of the pine oil, oleic acid, and derris resins caused no interference in the method. The third column gives the percentage of pyrethrins determined by the mercury reduction method on the solution that had been titrated by the Seil method, and shows that part of the titration in that method is due to volatile acids, other than the chrysanthemum monocarboxylic acid.

In commenting on the results in Tables 1 and 2 Holaday stated that the mercury reduction method and the Seil method give comparable results on samples that contain no interfering substances, but that an appreciable quantity of the acids titrated in the Seil method is not the

[1] Contributions from Boyce Thompson Institute, 8, No. 3, 175-181 (1936).
[2] *Ind. Eng. Chem. Anal. Ed.*, 10, 5 (1938).

chrysanthemum monocarboxylic acid. This error is in the opposite direction to that caused by the incomplete recovery of the chrysanthemum monocarboxylic acid in the steam distillation in this method and is therefore compensatory to it.

TABLE 2.—*Pyrethrin I in mineral oil-pyrethrum extracts*

MATERIAL ANALYZED	MERCURY REDUCTION METHOD	SEIL METHOD	MERCURY REDUCTION ON TITRATED SOLUTION FROM SEIL METHOD
	per cent	*per cent*	*per cent*
Official control insecticide*	0.044	0.042	—
	0.044	0.041	—
Official control insecticide+1% methyl salicylate	0.047	0.050	0.043
	0.047	0.054	—
Official control insecticide+2.5% β butoxy-β′ thiocyanodiethyl ether	0.044	—	—
	0.045	—	—

* Prepared by the National Association of Insecticide and Disinfectant Manufacturers. Analysis by the Referee gave 0.045 by both the mercury reduction method and the Seil method.

In mineral oil sprays containing methyl salicylate, it is difficult to remove all of this ingredient by the preliminary steam distillation required in the Seil method, and furthermore there is a loss of pyrethrins during the distillation.

Although these errors are compensating, the results in Table 2 show that the presence of methyl salicylate causes high results by the Seil method.

The mercury reduction method as modified by Holaday seems to be specific for Pyrethrin I and is not affected by the usual ingredients of pyrethrum spray materials.

DERRIS AND CUBE POWDER

The Referee has been investigating methods for the determination of rotenone in derris and cube powder in collaboration with H. A. Jones of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. In view of this fact no general collaboration was sought. This work is described in *Ind. Eng. Chem. Anal. Ed.*, **10**, 19 (1938); and *This Journal*, **21**, 148 (1938).

The results in Table 3 were obtained by Jones and Graham on a series of samples of derris and cube by this method. For comparison there are also included in the table the results obtained by multiple extraction with boiling benzene. In the latter method the sample was boiled under a reflux condenser with 200–300 cc. of benzene for 1–2 hours and filtered by suction. The marc was washed with hot benzene and then boiled again

with a fresh portion of solvent, followed by filtering and washing. This procedure was repeated three times, after which the Durham color test showed that the rotenone had either been completely extracted or reduced to a mere trace.

TABLE 3.—*Rotenone in derris and cube powder*
(Results expressed as percentages)

SAMPLE	CHLOROFORM EXTRACTION METHOD			BOILING BENZENE MULTIPLE EXTRACTION METHOD
	ANALYST, JONES	ANALYST, GRAHAM	AVERAGE	
2120-Derris	8 0	8.0	8 0	7 5
2121-Derris	10.0	10 4	10.2	—
3001-Derris	5.2	5.5	5.4	5.4
3002 Derris	1.9	2.1	2.0	2.0
3006-Derris	3 6	3.6	3.6	3.7
3007-Derris	0.5	0.6	0.6	—
3126-Derris	5.7	5.9	5.8	5.8
3307-Derris	7.2	7.5	7.4	7.1
3354-Derris	0.7	0 6	0 6	—
3355-Derris	3 2	3 6	3.4	3.4
2119-Cube	5.0	5.1	5 0	4.7
2665-Cube	2.4	2.3	2.4	—
2801-Cube	3.6	3.9	3.8	—
3004-Cube	2.8	3.0	2.9	2.8
3005-Cube	5.4	5 7	5.6	5 6
3230-Timbo	3.8	4.0	3.9	3.6
3260-Timbo	4.3	4.4	4 4	4.6
3107-Cracca	1 3	1.4	1.4	—

The Durham test could not be used on the marc from the chloroform extraction method owing to the fact that it was saturated with the extract. However, since the results by this method agree very well with those by the benzene, multiple extraction method, it may be assumed that extraction was fairly complete.

RECOMMENDATIONS[1]

It is recommended—

(1) That the mercury reduction method for the determination of Pyrethrin I in pyrethrum powder and mineral oil-pyrethrum extracts be adopted as a tentative method, and that a study of the method be continued.

(2) That the chloroform extraction method for the determination of rotenone in derris and cube powder be adopted as a tentative method and that a study of the method be continued.

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 59 (1938).

REPORT ON NAPHTHALENE IN POULTRY LICE PRODUCTS

By R. JINKINS (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

No previous official study has been made on methods for determining naphthalene quantitatively.

This year the Associate Referee limited his efforts to application of available methods to pure naphthalene. Available literature on the subject shows three different methods for determining naphthalene: sublimation,[1] picric acid,[2] and oxidation to phthalic acid.[3] Determinations were made by variations of the methods of sublimation and of picric acid precipitation, as well as by bromination, which involves the addition of four bromine atoms.[4] A brief account of the procedures used and the results obtained follows:

1. *Sublimation of pure naphthalene with Hortvet sublimator at reduced pressure.*—The results obtained by difference in weight of sublimator on a .50 gram sample gave recoveries of naphthalene of 98.8, 100, and 100.5 per cent.

Results were also obtained by means of the Hortvet sublimator on samples similar to lice powders, prepared in the laboratory. The samples contained naphthalene, 30 per cent; sulfur, 10 per cent; cresol, 3 per cent; and talc, 57 per cent.

On a 1.00 gram sample, sublimed under reduced pressure for periods of time varying from 1.5 to 3 hours, and temperature varying from 75° to 100° C., the recoveries of naphthalene ranged from 68 to 113.8 per cent. The higher results were obtained when the higher temperatures were used, and some showed sulfur in the sublimate.

2. *Picric acid precipitation.*—This method was attempted by R. D. Stanley of this Station, who, by treating known amounts of pure naphthalene with picric acid and titrating the excess picric acid in the filtrate after removal of the naphthalene picrate, obtained variable precentage recoveries, some as high as 97 per cent.

3. *Bromination.*—(a) The U.S.P.XI method for phenol was tried on a 0.05 gram sample, and recoveries of 86.2 and 93.6 per cent were obtained. (b) A variation of the procedure[5]—5 cc. of chloroform was used as solvent, and the time of bromination was 30 minutes with agitation. The mixture was then allowed to stand 15 minutes, after which 5 cc. of 40 per cent potassium iodide solution was added. When a 0.05 gram sample of pure naphthalene was used the recoveries varied in twelve determinations

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[1] Wiley's Principles and Practice of Agricultural Analysis, Vol. II, 3rd ed., 1931, p. 608. Also Hoffmann and Johnson, *This Journal*, 13, 367 (1930).
[2] Binnington and Geddes, *Ind. Eng. Chem. Anal. Ed.*, 6, 461 (1934).
[3] Miller, *This Journal*, 17, 308 (1934).
[4] Remsen's Organic Chemistry.
[5] Day and Taggart, *Ind. Eng. Chem.*, 20, 545 (1928).

between 89.8 per cent and 98.4 per cent, the average being 94.5 per cent. The time of bromination only was then varied to 1, 5, 10, and 15 minutes, respectively. Recoveries ranged from 21.0 to 66.8 per cent. With 1 hour, 1.5 hours, 2 hours, and 3 hours, the recoveries were from 93.3 to 113.2 per cent.

Bromination was continued for 30 minutes, with agitation, then the mixture was allowed to stand 15 minutes, after which 5 cc. of 40 per cent potassium iodide solution was added, with 1.0 cc. of carbon tetrachloride as solvent. Recoveries were from 94 to 106.4 per cent.

With 2 hours of bromination with frequent agitation, and 5 cc. of carbon tetrachloride used as solvent, the recoveries were from 70.0 to 119.2 per cent. With 2 hours of bromination with frequent agitation and 5 cc. of chloroform used as solvent, recoveries of 76.8 to 77.4 per cent were obtained.

As sufficient work has not been done on this problem and the results obtained are rather unsatisfactory, no specific recommendation for the future study of any one particular method can be made. It is recommended,[1] therefore, that the study be continued next year.

REPORT ON DISINFECTANTS

By C. M. Brewer (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The phenol coefficient method of testing disinfectants, *Methods of Analysis, A.O.A.C.*, 1935, 68, is the F. D. A. test. It is prescribed by the Secretary of Agriculture for use, where applicable, in the examination of antiseptics and disinfectants in the enforcement of the Insecticide Act of 1910. In June, 1933, the National Association of Insecticide and Disinfectant Manufacturers formally adopted the F. D. A. test as the official method for its membership.

At last year's meeting of this Association the Referee gave a brief unwritten report on the extensive use that this method now enjoys. The advantages of the method and the reasons for its very general use have been given elsewhere on numerous occasions. The chief adverse criticism that can be made of the test is its latitude of experimental error as compared with most chemical tests. This criticism, however, is applicable to all biological tests, but less so to this test than to any other bacteriological method for testing disinfectants. C. C. Baird, President of Baird and McGuire, Inc., and Past President of the National Association of Insecticide and Disinfectant Manufacturers, made the following statement in *Soap*, 9, (2), 89 (1933): "During the past year we have made a number of comparative determinations in which the R. W. method, the H. L.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 59 (1938).

method and the F. D. A. method were employed in determining the phenol coefficients of coal tar disinfectants.... Our bacteriologists have convinced us that the F. D. A. test is not only more simple to perform but the results obtained are more accurate...."

The voluminous amount of testing that has been done by the method, which has extended for more than 20 years in the enforcement of the Federal Insecticide Act, has convinced those familiar with this type of work of its desirability and dependability. In addition, there was a definite collaborative project undertaken in 1933 to determine its efficiency. This project was conducted by William Dreyfus, Chairman, Committee on Standardization of Disinfectants, National Association of Insecticide and Disinfectant Manufacturers. Details are reported in *Soap* **10** (1), 91 (1934). Two laboratories participated, Pease Laboratories, New York, and Skinner and Sherman, Inc. In his report Dreyfus states in part: "It is very gratifying to your Committee that on the whole the results agree remarkably well by using the three methods on all five samples, particularly in the case of the F. D. A. and the Hy Lab methods. . . . The results of all four of the coal tar disinfectants which were tested by the two laboratories against *Staphylococcus-Aureus* by the F. D. A. method at 20° C. show absolutely uniform results. . . . In conclusion I feel sure this report gives you a picture of the enormous amount of painstaking work undertaken by the investigators, Messrs. Gray and Philbrick, extending over a period of about six months, necessitating several hundred individual tests. . . . "

In view of the very general use made of the method for testing disinfectants, including its approval by those long experienced in disinfectant work, the Referee recommends[1] that the F. D. A. phenol coefficient test be adopted by this Association as an official method.

REPORT ON SUGARS AND SUGAR PRODUCTS

By C. A. Browne (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

The report of the Referee on Sugars and Sugar Products for the present year is largely a progress report and will be brief. Reports by associate referees on honey; drying, densimetric, and refractometric methods; chemical methods for reducing sugars; lead precipitate; and acetylmethyl carbinol and diacetyl in food products will be submitted, but communications on maple products, polariscopic methods, unfermentable sugars in molasses, refractive indices of sugar solutions, and other unreported subjects are deferred until the completion of work that is at present under way. As an offset to these gaps in the program for this year, a

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 64 (1938).

paper will be presented by Max Phillips of the Bureau of Chemistry and Soils on "The Effect of Various Carbohydrate Materials on the Determination of Lignin by the Fuming Hydrochloric Acid Method." See this Journal, **21**, 140 (1938).

Your Referee approves of the various recommendations made by his associates but would make one slight reservation with regard to the immediate dropping of the official Wein method for the determination of maltose. The work of our referees and their collaborators has a threefold purpose, first to improve existing methods of analyses, second to try out promising new methods for analyzing agricultural products, and third to eliminate old methods that have outgrown their usefulness. As a body of official analysts the Association has erred at times, but on the safe conservative side, in retaining methods beyond the time when they should have been dropped. It is surprising to note, however, how tenaciously some analysts cling to certain obsolete methods either from long habit or from unwillingness to depart from established routine. Our *Methods of Analysis* must be subjected to a constant pruning away of useless material if it is to maintain a vigorous growth. There should be a safeguard, however, against too hasty a scrapping of old work, and for this reason if an established official method is recommended by a referee for deletion it would seem desirable, as in the adoption of new official methods, that such a recommendation go over to a subsequent meeting for a second reading before final deletion. Such a procedure is implied, although not definitely stated, in the present by-laws. Deferring action in this way will give sufficient time for a full consideration of the matter, especially by absent members, who may not have been present when the recommendation for rejection of an official method was first introduced.

Because of other duties your present Referee on Sugars and Sugar Products, after three years of service in this old capacity, desires to hand over this work to a younger colleague, who can take the time to prepare samples and to secure the necessary collaboration upon which the success of this work depends.

REPORT ON HONEY—DETERMINATION OF LEVULOSE

By R. E. Lothrop (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

During the past year the Associate Referee on Honey has devoted his attention to the tentative method for the determination of levulose in honey, which is based on the change in the rotatory power of levulose when polarized at different temperatures. The change in the optical rotation of other bodies associated with levulose in honey is so little that it affects the results to only a slight degree. The method has the advantage of being quite rapid, provided suitable equipment for high temperature polarization is available.

The method as given in *Methods of Analysis, A.O.A.C.*, 1935, page 488, is the one used by Browne for the analysis of honey as reported in Bureau of Chemistry Bulletin 110, published in 1908. It is referred to by Browne in this bulletin as the Wiley optical method for estimating levulose because it was devised by Wiley and first described by him in an article entitled "On Estimation of Levulose in Honeys and Other Substances."[1] The method also appears in Wiley's *Principles and Practice of Agricultural Analysis*, vol. III, p. 267 (1897).

In the method described by Wiley, it is shown that 1 gram of levulose in 100 cc. gives a variation of 0.0357° V. for each degree Centigrade change in temperature when polarized in a 200 mm. tube and that the variation is uniform between 0° and 88°. The variation for 67° (the difference between 20° and 87°) would, therefore, be 0.0357×67, or 2.3919. The difference in direct polarization of a honey at 20° and 87° C. divided by 2.3919 will give, therefore, the grams of levulose in a normal weight of honey, from which the percentage of levulose is easily calculated. This same factor, namely 2.3919, is the one given in the present tentative method.

The history of this method is traced here to emphasize the long period of time that has elapsed since its introduction without any changes being made in it. Better methods of purifying levulose have since been introduced, resulting probably in somewhat purer preparations for experimental use. New technic for high temperature polarization has also been introduced. These facts seem to justify an investigation of the value 0.0357, which represents the change in rotation of 1 gram of levulose per 100 cc. for each degree Centigrade when the solution is polarized in a 200 mm. tube.

In a recent paper published by Jackson and Mathews, entitled "Some Physical Properties of Levulose and Its Estimation by Copper Reduction Methods,"[2] the value for $\Delta P/\Delta T \times g$ was found to be 0.03441 instead of 0.0357, the value given in the tentative method. Determinations were made at concentrations from 3 to 18 grams per 100 cc., and the value for $\Delta P/\Delta T \times g$ did not show any systematic variation between these concentrations. When this value is used in calculating levulose in honey, results from 1.5 to 2.0 per cent higher are obtained than when the old factor is used.

Comparative values for the levulose content of ten different honeys determined iodometrically and by the tentative method (polarization at high and low temperatures) are given in an article, entitled "Determination of Dextrose and Levulose in Honey by Use of Iodine Oxidation Method," by Lothrop and Holmes.[3] The average value for levulose in

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[1] *J. Am. Chem. Soc.*, 18, 81 (1896).
[2] Research Paper No. 426, *Bur. Standards J. Research*, Vol. 8, March, 1932.
[3] *Ind. Eng. Chem. Anal. Ed.*, 3, 334 (1931).

ten samples of honey by the polarization method was found to be 38.70 per cent, and by the iodometric method 40.03, or an average difference between the two methods of 1.33 per cent.

If the Jackson and Mathews factor for $\Delta P/\Delta T \times g$ of 0.03441 be used in place of the old factor 0.0357, the average value for the levulose content of these 10 samples of honey by polarization becomes 40.22 per cent, instead of 38.70, which differs by only 0.19 per cent from the average value determined iodometrically.

Since the ratio of dextrose to levulose in honey is one of the criteria used in detecting adulteration with commercial invert sugar, any step to increase the accuracy of the method of determining this ratio would be desirable.

In the following work, a preparation of highly purified levulose was used; it gave the specific rotation, $\alpha_{20}^{D}=91.65$. This value agrees very closely with Vosberg's rotation[1] for levulose calculated to this temperature and concentration.

Solutions containing, respectively, 8.7135 grams and 12.6630 grams of levulose per 100 cc., were made up at 20° C. and polarized at approximately 20° C. and 70° C., 400 mm. monel polariscope tubes of special design being used. In this way a difference of about 50° C. was obtained between low and high temperature polarizations. $\Delta P/\Delta T \times g$ values obtained were 0.0347 and 0.0336, respectively, or an average value of 0.03415, which compares with 0.03441 for the value obtained by Jackson and Mathews, and 0.0357 for the tentative method. Similar polarizations conducted with the same levulose solutions, to which approximately equal amounts of pure dextrose had been added, did not change the values of $\Delta P/\Delta T \times g$ to a significant extent.

In view of these results, it is recommended[2] that a collaborative study be made of this method, particularly of the value of $\Delta P/\Delta T \times g$ for pure levulose, with a view to increasing the accuracy of the high and low temperature polarization method of determining levulose in honey.

No report on maple products was given by the associate referee.

REPORT ON DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS

By CARL F. SNYDER (National Bureau of Standards, Washington, D. C.), *Associate Referee*

The report presented by the Associate Referee at the 1936 meeting of the Association was not published, nor were the recommendations acted

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[1] *J. Am. Chem. Soc*, 42, 1696 (1920).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 72 (1938).

upon, due to some misunderstanding. Therefore the same report and recommendations are presented at this time for action.

MOISTURE

In *Methods of Analysis, A.O.A.C.*, 1935, 462, three official drying methods are given. The first, **2**, is a method of direct drying at atmospheric pressure at the temperature of boiling water. This method is applicable to raw cane and beet sugars and refined sugar. The other two methods specify drying on specially prepared pumice or quartz sand at 70° C. under a pressure of not to exceed 100 mm. of mercury. These methods are applicable to massecuites, molasses, and other liquid and semiliquid products.

At the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis, held in London, August 31 to September 5, 1936, the report on drying methods was presented by the referee on this subject, H. C. S. de Whalley. The methods in use in the various countries were the subject of study. Incorporated in this report was a communication from Associate Referee R. T. Balch of the Bureau of Chemistry and Soils, which stated in part:

> Distinction should be made of the accuracy of results it is wished to attain. For referee or investigational work of highest character it is essential that drying of all samples be conducted in a vacuum chamber, whereas, if accuracy must be sacrificed for speed, as is frequently the case in factory control work, the drying may be conducted at atmospheric pressures and higher temperatures. In my opinion the determination of moisture in a vacuum must be considered the standard, and the temperature and pressure should not exceed 70° C. and 5 cm. pressure, respectively; also the vacuum chamber should be bled with dry air to insure the removal of the water vapors during the drying operation.

The International Commission for Uniform Methods of Sugar Analysis adopted the vacuum drying method for refined sugars and normal raws, specifying the temperature 60° and a pressure not exceeding 5 cm. of mercury.

It is therefore recommended that this method, which was published in *This Journal*, **21**, 89 (1938), be adopted by this Association.

SOLIDS BY MEANS OF REFRACTOMETER

The Associate Referee has already called attention to the need of eliminating the break in the so-called Schönrock-Main table of refractive indices of sucrose solutions. This table contains both the Schönrock and the Main values of the refractive index for 66 per cent sucrose solution, which separate values differ by three units in the fourth decimal place. In order to eliminate this difference these values were plotted on a large-scale graph, the Schönrock from 34 to 38 per cent water and the Main from 30 to 38 per cent water. If the methods employed by the two investigators are considered, it is evident that the method of Schönrock is capable of higher precision than is possible in the direct readings of the

Abbé refractometer of Main. Therefore the curve of the Schönrock values was extended as a straight line from 34 per cent water to meet the Main curve at 30 per cent water. Values of the refractive indices were taken from this extended curve.

A table was then constructed by E. Landt[1] consisting of the five-place 1933 Schönrock-Landt values from 0 to 24 per cent sucrose, the four-place 1911 Schönrock values from 24 to 66 per cent sucrose, the extrapolated values from 66 to 70 per cent sucrose, and the four-place Main values from 70 to 85 per cent sucrose. This table was adopted by the International Commission for Uniform Methods of Sugar Analysis, and is designated the "International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936." A comparison of the temperature correction values of Schönrock and of Stanek led to the conclusion that the former were to be preferred, *This Journal*, **18**, 171 (1935). The International Commission for Uniform Methods of Sugar Analysis adopted a table of temperature corrections based on the values of Schönrock.[2] This table, which replaces the Stanek temperature correction table, is designated "International Temperature Correction Table, 1936."[3]

Methods of Analysis, A.O.A.C., 1935, contains a temperature table, page 624, essentially the same as the International Temperature Correction Table, and a table of indices of sucrose solutions, page 622, which differs from the International Scale only in that the values from 0 to 24 per cent sucrose are given to four places only.

RECOMMENDATIONS[4]

It is recommended—

(1) That the International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936, be adopted as official (first action).

(2) That the International Temperature Correction Table, 1936, be adopted as official (first action).

(3) That the vacuum drying method for the determination of moisture in cane and beet raws and refined sugars, be adopted as official (first action).

No report on polariscopic methods was given by the associate referee.

REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS

By R. F. Jackson (National Bureau of Standards, Washington, D. C.), *Associate Referee*

A preliminary investigation has been made of Shaffer and Hartmann's macromethod for reducing sugars. The method recommends itself for

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[1] *Deut. Zuckerind.*, 61, 997, 1026 (1936).
[2] *Z. Vereins Deut. Zuckerind.*, 61, 425 (1911).
[3] E. Landt, *Deut. Zuckerind.*, 61, 1027 (1936).
[4] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 72 (1938).

study because of its convenience and rapidity, because the mildly alkaline solutions of which it is composed attack "nonreducing" constituents of the sample less strongly than do those solutions which contain caustic alkali, and because the delicate iodometric end point of the titration appears to promise a satisfactory analytical precision.

A considerable number of analyses of dextrose, levulose, invert sugar, and of invert sugar-sucrose mixtures was made, but since in no instance was the series complete it is deemed advisable to postpone publication of results until complete data can be presented.

It is recommended[1] that Wein's method for maltose, *Methods of Analysis, A.O.A.C.*, 484, **54** and **55**, and Table **12**, p. 634 be discarded.

ARGUMENT

1. The method, originally described in the early period of development of sugar analysis, is now obsolete, having been superseded by the more modern unified methods.

2. The procedure of Wein duplicates closely that of Munson and Walker. The same copper reagents are used in both methods, but Wein specifies a total volume of solution of 75 cc., while Munson and Walker employ a volume of 100 cc. Wein specifies a boiling period of 4 minutes, while Munson and Walker require a 2 minute period of boiling. In spite of the longer period of boiling specified by Wein, the copper-sugar equivalents are nearly the same as those of Munson and Walker. Thus calculated to anhydrous maltose:

TABLE 1.—*Copper-sugar equivalents*

MALTOSE	COPPER	
	WEIN	M. & W.
mg.	*mg*	*mg.*
100	115.1	114.5
200	226.7	226.5
250	282.5	282.7

As appears from Table 1, Munson and Walker obtain a greater weight of copper from 250 mg. of maltose in 2 minutes than Wein obtains in 4 minutes.

Either the greater alkalinity of Wein's reagent diminishes the amount of copper reduced, an effect shown by Quisumbing and Thomas,[2] or Wein's table is in error, as claimed by Brown, Morris, and Miller, and by Ling and Baker (see Browne's Handbook of Sugar Analysis, p. 423). If it is necessary to revise the copper tables, it would seem advisable to concentrate on Munson and Walker's values.

[1] For report of Subcommittee D and action of the Association, see *This Journal*, **21**, 72 (1938).
[2] *J. Am Chem Soc.*, **43**, 1503 (1921); *This Journal*, **15**, 79 (1932).

3. Wein's method is applicable solely to pure maltose, which is of relatively rare occurrence. Almost all maltose products contain dextrose and other sugars, and an analysis can properly be conducted only by the unified methods, such as those of Munson and Walker, Lane and Eynon, and for small samples, Scales. In order to make Wein's method available for sugar mixtures it would be necessary to determine the copper-sugar equivalents for all the sugars. This seems inadvisable.

4. Munson and Walker's tables include a range of maltose concentrations up to 406 mg., while Wein's table extends only to 265 mg.

5. In view of the fact that disaccharides reduce copper more slowly than do monosaccharides, it remains to be determined whether precision of analysis is sacrificed by selecting a 2 minute period of boiling instead of a 4 minute period. Five reductions by 50 cc. portions of solution, each containing 325.9 mg. of maltose hydrate (purity undetermined), were conducted by Munson and Walker's method, the time of boiling being varied from 1 to 4 minutes. The copper reduced is given in Table 2.

TABLE 2.—*Reduction by 325.9 mg. of maltose hydrate*

TIME	COPPER
Minutes	*mg.*
1	337.6
1.5	339.4
2	343.4
3	346.2
4	348 5

The results were least squared, yielding the equation $Cu=4.556m-0.214m^2+334.0$. This equation, differentiated with respect to time, showed that at the 2 minute point the precipitate was increasing at the rate of 3.7 mg. per minute, and at the 4 minute point at 2.9 mg. per minute. If the analyst made an error of 0.1 minute in judging the boiling time the error would be 0.08 mg. or 0.03 per cent greater in the shorter than in the longer time of boiling. For practical purposes Munson and Walker's procedure is satisfactory.

REPORT ON LEAD PRECIPITATE

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*

In accordance with the recommendations made last year, *This Journal*, **20,** 401 (1937), a larger number of raw sugar samples were clarified with mixtures of Horne's dry lead and dry mercuric acetate in varying proportions. Of the 50 samples used in the first series, 13 were from Cuba,

7 from Puerto Rico, 9 from the Philippines, and the remaining samples from miscellaneous sources supplying the refineries in the United States and Canada.

The experiments were carried out by C. A. Gamble and J. E. Mull of this Laboratory, as previously described, and the following results were obtained:

CLARIFYING AGENTS USED	AVERAGE POLARIZATION
gram *gram*	
None	97.099
0.2 dry lead, 0.1 dry mercuric acetate	97.168
0.2 dry lead, 0.2 dry mercuric acetate	97.152
0.2 dry lead, 0.3 dry mercuric acetate	97.131

The results confirm those previously obtained with a smaller number of samples. An increase in the quantity of mercuric acetate used in addition to the dry lead brings the polarization closer and closer to the true value, but even with 0.3 gram there is still an average plus difference of 0.032. It was concluded that it would probably be necessary to reduce the dry lead rather than further to increase the mercuric acetate. A second series of tests was therefore started, in which only 0.1 gram of dry lead was used together with increasing quantities of mercuric acetate. Eleven raw sugar samples from various sources were clarified in this manner with the following results:

CLARIFYING AGENTS USED	AVERAGE POLARIZATION
gram *gram*	
None	96.205
0.1 dry lead, 0.2 dry mercuric acetate	96.232
0.1 dry lead, 0.3 dry mercuric acetate	96.241
0.1 dry lead, 0.4 dry mercuric acetate	96.217

It is noted that the average deviations from the true polarization are very small, the largest (0.036) being of about the same order of magnitude as the smallest (0.032) found in the first series. With 0.1 gram of dry lead and 0.4 gram of mercuric acetate the deviation was only 0.012, and this is about as close an agreement as may be expected, although it appears that the dry lead might still be reduced a little further. Little would be gained by trying other mixtures because exact agreement between different series of experiments with sugars from other sources would undoubtedly give slight fluctuations up or down from the average true polarization.

Although a much closer approach to the true direct polarization of raw sugars could be obtained by clarification with the mixture of dry lead and dry mercuric acetate specified above, the method can not be recom-

mended for practical reasons. The dry mercuric acetate reacts very slowly with the solution and tends to form a ball, which is very difficult to disintegrate by shaking. The total precipitate is very small in volume and it does not filter so well as the more voluminous precipitate obtained with the usual quantity of lead subacetate alone. The method is therefore not applicable for routine work, but it may be found of value in certain research problems.

It is recommended[1] that the study of the lead precipitate be discontinued for the present.

REPORT ON ACETYL-METHYL CARBINOL AND DIACETYL IN FOOD PRODUCTS

By John B. Wilson (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Since Browne[2] reported the presence of acetylmethylcarbinol in deteriorated vinegar, this substance has been found in a variety of products. Later he also demonstrated its presence in fermented sugar cane juice.[3] Balcom[4] showed acetylmethylcarbinol to be a normal constituent of vinegar. Harden and Walpole[5] found it in cultures of *B. lactis aerogenes*, while van Niel, Kluyver, and Derx[6] found it in butter and in buttermilk, in which it is important as the source of diacetyl, a constituent of the aroma of butter.

Schmalfuss and Barthmeyer[7] found diacetyl in milk cultures and cultures of lactic acid bacteria. Later, these authors[8] reported diacetyl in butter, honey, beer, coffee, cacao, and tobacco smoke, while more recently[9] they found that these products contain acetylmethylcarbinol as well as diacetyl. During the past year the Associate Referee was informed by a correspondent that the first fractions of the alcohol obtained by fermenting cane molasses contain diacetyl, but that as yet no trace of acetylmethylcarbinol has been found in molasses alcohol.

Although Browne and Balcom identified acetylmethylcarbinol as phenyldiacetylosazone, the majority of workers have oxidized it to diacetyl by adding ferric chloride solution before distilling, and subsequently have made a precipitation of nickel dimethylglyoxime for its determination.

For some time it has been known that this salt is a reliable precipitant for the determination of nickel, and van Niel[10] has shown that dimethyl-

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[1] For report of Subcommittee D and action of the Association, see *This Journal*, 21, 72 (1938)
[2] *J. Am. Chem. Soc.*, 25, 16 (1903).
[3] *Ibid.*, 28, 468 (1906).
[4] *Ibid.*, 39, 309 (1917).
[5] *Proc. Roy Soc.* Series B., 77, 399 (1906).
[6] *Biochem. Z.*, 210, 234 (1929).
[7] *Z. Physiol. Chem.*, 176, 282 (1928).
[8] *Biochem. Z.*, 216, 333 (1929).
[9] *Z. Unters. Lebensm.*, 63, 283 (1932).
[10] *Biochem. Z.*, 187, 472 (1927).

glyoxime is precipitated quantitatively by an excess of nickel chloride in a solution containing sodium acetate. He also obtained approximately quantitative results when 58 mg. of diacetyl was precipitated with hydroxylamine and nickel chloride solutions when heated to 80° or 90° C. for 1 or 2 hours. The average yield on seven samples containing about 25 mg. of acetylmethylcarbinol was 97 per cent when distilled with ferric chloride and precipitated as the nickel salt in the distillate.

Olivier[1] devised a procedure for purifying diacetyl by means of a crystalline compound with orthophosphoric acid and recommended the use of van Niel's method with one exception—that the mixture be heated on the water bath for 2 hours instead of 1 hour.

Barnicoat[2] has made an extensive study of the van Niel method when applied to quantities of diacetyl of 10 mg. or less and gives a procedure that yields good results with these quantities. He did experience difficulty, however, since in some cases the precipitation of the nickel dimethylglyoxime was not complete, further small quantities being precipitated when the filtrates were evaporated to a small volume and permitted to stand. This behavior is attributed to slowness in the reaction with the second hydroxylamine molecule, since the immediate disappearance of the odor and color of diacetyl upon the addition of hydroxylamine has led to the conclusion that the first molecule reacts very quickly.

The Associate Referee also experienced difficulty in obtaining complete precipitation of diacetyl as nickel dimethylglyoxime when he used approximately 93 mg. portions of diacetyl in 100 cc. of solution. The low results are probably due to polymerization of diacetyl since results of the same order were obtained on the same stock solution with other precipitating reagents, such as phenylhydrazine, semi-carbazide, *p*-nitrophenylhydrazine, and thiosemicarbazide, although an insufficient amount of work was done with these precipitants to estimate their suitability for use with diacetyl. Further evidence in favor of the polymerization theory lies in the fact that the diacetyl content of the stock solution, as measured by precipitation as nickel dimethylglyoxime, decreased with the age of the solution. In every case, however, more precipitate was obtained by evaporating the filtrate to dryness and taking up in a small volume of water. In some cases a further quantity of hydroxylamine was added to the second filtrate, and upon evaporation further quantities of the red precipitate were obtained.

With a view to increasing the yield of nickel dimethylglyoxime, the Associate Referee made about fifty precipitations, varying the conditions somewhat. The solution was buffered by the addition of ammonia, sodium acetate, and ammonium chloride. The time of heating was varied. Ammonia, sodium carbonate, and sodium hydroxide were used to free the hydroxylamine from the chloride both before and after the reagent had

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[1] *Bull. Soc. Chim.*, 51, 99 (1932).
[2] *Analyst*, 60, 653 (1935)

been added to the diacetyl solution. The nickel salt was added at various stages of the procedure, and the excess of hydroxylamine was varied. In other experiments an excess of alkali was present during the treatment with hydroxylamine, and the solution was neutralized before the addition of the nickel salt. None of these modifications had the desired effect of 100 per cent yield in one precipitation.

Kniphorst and Kruisheer[1] recommend the precipitation of nickel dimethylglyoxime as a means of determining 2,3-butyleneglycol, and while they give no estimate of the yield in the case of diacetyl, they state that they obtained 96 per cent yield for acetylmethylcarbinol and 93 per cent yield for 2,3-butyleneglycol.

In addition to the gravimetric methods a number of colorimetric methods have been recommended for determination of diacetyl. Barnicoat[2] uses the nickel dimethylglyoxime for a colorimetric procedure by dissolving 1 mg. or less of precipitate in chloroform and comparing with a standard similarly treated.

The creatine test has been used qualitatively by Hammer[3] and others. Pien, Baisse, and Martin[4] give a method depending upon the reaction of diacetyl with orthodiamines to form quinoxalines, and still more recently[5] a method for diacetyl in butter depending upon a condensation with diamino benzidine.

During the coming year the Associate Referee plans to apply the nickel dimethylglyoxime method to freshly distilled diacetyl solutions obtained by mixing known quantities of dimethylglyoxime with acid and distilling into hydroxylamine solution to prevent the polymerization of diacetyl. If the results obtained warrant, the procedure will be applied to distillates from acetylmethylcarbinol in the presence of oxidizing agents and later to natural products containing diacetyl and/or acetylmethylcarbinol and/or 2,3-butyleneglycol.

It is planned also to ascertain the applicability of the colorimetric procedures mentioned previously to quantities of diacetyl, acetylmethyl carbinol, and 2,3-butylene glycol in quantities of 1 mg or less.

It is recommended that the study of methods for determining acetylmethylcarbinol and diacetyl in food products be continued.

No report on unfermentable sugars in molasses was given by the associate referee.

No report on refractive indices of sugar solutions was given by the associate referee.

No report on vinegars was given by the referee.

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[1] *Z. Unters. Lebensm.*, 73, 1 (1937).
[2] *Loc. cit.*
[3] *J. Dairy Sci.*, 18, 579 (1935).
[4] *Ann. fals.*, 98, 204 (1936).
[5] *Lait.* 17, 673 (1937).

REPORT ON ASH IN VINEGAR

By HARRY SHUMAN (U. S. Food and Drug Administration, Philadelphia, Pa.), *Associate Referee*

Last year it was pointed out that the ash of cider vinegar is hygroscopic and is liable to pick up considerable moisture in the course of desiccation and weighing. It was also demonstrated that soluble and insoluble P_2O_5 determinations were empirical in that they varied with temperature of ashing. Therefore, Subcommittee C has recommended that studies be continued with a view to standardizing the directions. It was also recommended that method **58** (a), p. 456, *Methods of Analysis, A.O.A.C.*, 1935, be studied with a view to defining more closely temperature and time factors.

Two samples of cider vinegar were sent to collaborators with the following directions for analysis.

ASH

Measure 25 cc. of the vinegar into a weighed platinum dish, evaporate to dryness on a steam bath, and heat in a muffle at 500° C. for 30 minutes. (Cover floor of muffle with a sheet of 3/16″ or ¼″ asbestos to avoid overheating ash due to the uneven temperature.) Break up the charred mass and exhaust with hot water, collect the insoluble residue on an ashless filter, and burn the filter and contents at 500° C. for 30 minutes, or until all the carbon is burned off. Add the filtrate, evaporate to dryness, and heat at 500° C. for 15 minutes. Cool in a desiccator and weigh (weight "A"). Reheat in muffle for 5 minutes, and cool in a desiccator containing a fresh, efficient desiccant (sulfuric acid is preferable, anhydrous calcium chloride or calcium carbide may be used). Place only one dish in a desiccator. Place weight "A" on balance pan before removing dish from desiccator, and weigh rapidly to nearest milligram (weight "B").

SOLUBLE AND INSOLUBLE ASH

Determine as directed in *Methods of Analysis, A.O.A.C.*, 1935, 456, **59**.

SOLUBLE AND INSOLUBLE PHOSPHORIC ACID

Determine as directed in *Methods of Analysis, A.O.A.C.*, 1935, 456, **61**, **62**.

It will be noted that the following three precautions, essential to the exclusion of moisture from the ash, are included in the collaborative procedure.

(1) Use of an efficient desiccant.

(2) Limited number of dishes in any one desiccator. Data in last year's report and additional observation since show that the number of dishes placed in one desiccator should not exceed two, because the repeated admission of air to a desiccator during the removal of dishes for weighing may add appreciable moisture to the remaining ashes.

(3) Rapid weighing. The first weighing, "A," is relatively slow and may include appreciable moisture, particularly if the moisture content of the air is high, as may be the case during the warm months of the year. Weight "B," however, is obtained in a minimum of time.

The collaborative results are given in the table. The values for total

Collaborative results on ash

COLLABORATOR	DESICCANT USED	MUFFLE TEMP. AUTOMATICALLY CONTROLLED	CIDER VINEGAR NO. 1 TOTAL ASH "A"	TOTAL ASH "B"	SOLUBLE ASH	INSOLUBLE ASH	SOLUBLE P_2O_5	INSOLUBLE P_2O_5	TOTAL P_2O_5 (SOLUBLE + INSOLUBLE)	CIDER VINEGAR NO. 2 TOTAL ASH "A"	TOTAL ASH "B"	SOLUBLE ASH	INSOLUBLE ASH	SOLUBLE P_2O_5	INSOLUBLE P_2O_5	TOTAL P_2O_5 (SOLUBLE + INSOLUBLE)
			g./100 cc.	g./100 cc.	g./100 cc.	g./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	g./100 cc.	g./100 cc.	g./100 cc.	g./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.
1	H_2SO_4	Yes	0.25 0.25 (0.25) (0.26)	0.24 0.24[a] (0.23) (0.23)[b]						0.32 0.30[a] (0.31) (0.30)[b]	0.29 0.29[a] (0.29) (0.29)[b]					
2	H_2SO_4	No[c]	0.25 0.25	0.24 0.24	0.20 0.21	0.036 0.032	7.5 7.3	8.8 9.1	16.3 16.4	0.31 0.30	0.30 0.30	0.26 0.26	0.036 0.036	8.4 8.7	10.0 —	18.4 —
3	H_2SO_4	Yes	0.26 0.25	0.25 0.25	0.22 0.22	0.033 0.030	6.3 6.4	7.6 7.6	13.9 14.0	0.31 0.32	0.31 0.31	0.27 0.27	0.036 0.036	7.7 7.7	7.3 7.3	15.0 15.4
4[d]	H_2SO_4	No	0.24 0.24	0.24 0.24	0.21 0.21	0.028 0.029	9.2 8.8	— 6.4	— 15.2	0.24 0.24	0.24 0.24	0.21 0.21	0.029 0.026	8.0 9.2	5.2 4.0	13.2 13.2
5	CaC_2	Yes	0.24 0.24	0.23 0.23	0.20 0.20	0.029 0.030	6.4 6.6	7.5 7.7	13.9 14.3	0.30 0.30	0.29 0.29	0.25 0.25	0.042 0.040	7.8 7.6	8.4 8.3	16.2 15.9
6	H_2SO_4[e]	No[f]	0.26 0.26 0.25	0.24 0.24 0.24	0.20 0.20 —	0.036 0.035 —	6.4 8.2 —	10.5 10.2 —	16.9 18.4 —	0.33 0.32 0.30	0.30 0.30 0.30	0.26 0.26 —	0.039 0.040 —	10.2 12.0 —	10.5 9.4 —	20.7 21.4 —
7	Anhy. $CaCl_2$	Yes	0.26 0.25	0.24 0.24	0.21 0.21	0.034 0.031	6.8 7.2	7.5 6.8	14.3 14.0	0.31 0.31	0.30 0.30	0.27 0.27	0.035 0.036	9.2 9.3	6.7 6.5	15.9 15.8
8	H_2SO_4	No[g]	0.25 0.24 0.25 0.24	0.24 0.24 0.25 0.25	0.20 0.20 0.21 0.21	0.042 0.037 0.040 0.035	— — 6.8 6.2	— — 7.9 8.6	— — 14.7 14.8	0.30 0.33 0.30 0.31	0.29 0.30 0.30 0.31	0.25 0.25 0.26 0.27	0.041 0.052 0.042 0.045	8.0 7.0 6.5 6.5	8.1 9.0 10.2 10.2	16.1 16.0 16.7 16.7
9	H_2SO_4	Yes	0.25 0.25	0.24 0.24	0.21 0.21	0.029 0.027	6.9 7.3	7.7 7.5	14.6 14.8	0.31 0.32	0.30 0.30	0.26 0.26	0.036 0.036	8.0 7.9	8.4 8.8	16.4 16.7
	Maximum Minimum Average		0.26 0.24 0.25	0.25 0.23 0.24	0.22 0.20 0.21	0.042 0.027 0.033	9.2 6.2 7.1	10.5 6.4 8.1	18.4 13.9 15.1	0.33 0.30 0.31	0.31 0.29 0.30	0.27 0.25 0.26	0.052 0.035 0.039	12.0 6.5 8.3	10.5 6.5 8.6	21.4 15.0 16.9

[a] By collaborative method.
[b] In muffle 4 hours before leaching, otherwise same as collaborative method.
[c] Highest temperature not over 520° C by pyrometer
[d] Results for vinegar No 2 not included in summary. See comments.
[e] Anhydrous $CaCl_2$ used for "A," H_2SO_4 for "B"
[f] Muffle temperature checked before and after ashing by pyrometer. Variation did not exceed ±10° C.
[g] Highest temperature not over 520° C by pyrometer.

ash, "B," are reasonably close for both vinegars, with the exception of one collaborator's results on vinegar No. 2. This suggests that this one result should be disregarded.

Although "A" results are almost as concordant as "B," and are on the average only 0.01 gram higher, it is believed that if samples had been analyzed by collaborators in July or August instead of in October, a greater variation among "A" results would be evident. In emphasizing the effectiveness of greater control in the ashing method it is recalled that the official ash methods for vinegars have been demonstrated to yield widely varying results. In the collaborative studies of two cider vinegars, Clarke, *This Journal*, **9**, 440 (1926); **10**, 490 (1927), obtained for Method **58 (a)** results ranging from 0.27 to 0.36 and 0.35 to 0.38 grams per 100 cc.; and for Method **58 (b)**, 0.27 to 0.32 and 0.34 to 0.40.

The varied results for P_2O_5 show that these determinations have not responded to standardization of ashing time and temperature alone. A consideration of the results for total P_2O_5 (soluble+insoluble) would suggest a probable variable loss of phosphoric acid during ashing; however, studies last year indicated that with cider vinegars loss of phosphoric acid is not significant. It appears, therefore, that other factors are responsible.

The Associate Referee wishes to express his appreciation to the heads of the collaborating laboratories and to the following chemists who did the analytical work (all members of the U. S. Food and Drug Administration with the exception of Mr. Laudig of the H. J. Heinz Co.):

1. F. Leslie Hart, Los Angeles.
2. Rupert Hyatt, Cincinnati.
3. L. A. Salinger, San Francisco.
4. C. A. Wood, New York.
5. L. W. Ferris, Buffalo.
6. J. F. Laudig, Pittsburgh.
7. D. A. Ballard, Seattle.
8. C. D. Schiffman, Atlanta.

RECOMMENDATIONS[1]

It is recommended—

(1) That the present official methods **58 (a)** and **58 (b)**, for the determination of ash in vinegars be dropped and that the method submitted by the Associate Referee be adopted. The method was published in *This Journal*, **21**, 89 (1938).

(2) That methods for the determination of total phosphoric acid in vinegars be studied.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 71 (1938).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

In 1936 the Referee reported work on the analysis of imitation vanilla in a vehicle of dilute glycerol, which indicated that the official gravimetric method for vanillin and coumarin gave fair results when applied to such a product and that the densimetric method for glycerol also showed promise of application to this type of product.

This year two imitation vanilla flavors were made up according to the formulas in Table 1.

TABLE 1.—*Formulas for and composition of imitation vanilla flavors*

FLAVOR	A	B	A	B
			per 100 cc.	*per 100 cc.*
Vanillin	2.50 g.	3.40 g.	0.50 g.	0.68 g.
Coumarin	0.80 g.	0.51 g.	0.16 g.	0.10 g.
Vanilla	30 cc.	30 cc.	6 cc.	6 cc.
Glycerol	200 g.	250 g.	32 cc.	40 cc.
Caramel	to color	to color		
Water	q.s.	q.s.		
Total	500 cc.	500 cc.		

As the glycerol used had a specific gravity of 1.2493 at 25°/25°, it was considered to be 100 per cent U. S. P. glycerol. The volume of glycerol in the flavors was obtained by dividing the weight used by the specific gravity.

The samples were analyzed by two collaborators for vanillin and coumarin by the official gravimetric method, *Methods of Analysis, A.O.A.C.*, 1935, 306, and for glycerol as described in last year's report, *This Journal*, **20**, 408 (1937). The results are given in Table 2.

TABLE 2.—*Imitation vanilla flavors in a vehicle of glycerol*

	JOHN B. WILSON		W. O. WINKLER		PRESENT	
	A	B	A	B	A	B
Glycerol per 100 cc. (cc.)	33.6	42.2	33.5	42.0	32.0	40.0
Vanillin per 100 cc. (gram)	0.50	0.68	0.50	0.70	0.50	0.68
Coumarin per 100 cc. (gram)	0.13	0.09	0.15	0.09	0.16	0 10

The results obtained this year corroborate those in last year's report regarding the determination of vanillin and coumarin in imitation vanilla flavors in a vehicle of glycerol.

In reviewing the results on the determination of glycerol obtained during the last two years, it will be noted that the discrepancy between the quantity of glycerol found and that actually present is fairly regular. The relationship between them is shown in Table 3.

TABLE 3.—*Relationship between glycerol found and that present in flavors*

GLYCEROL PRESENT	GLYCEROL FOUND	GLYCEROL FOUND / GLYCEROL PRESENT
cc./100 cc.	*cc./100 cc.*	
35.6	37.6	1.056
	37.6	1.056
32.0	33.6	1.050
	33.5	1.042
40.0	42.2	1.055
	42.0	1.050
		Av. 1.0515

The regularity shown in the error, which of course is due to the presence of soluble solids other than glycerol, suggests the possibility of the use of a correction factor.

It is recommended that the work on the determination of glycerol, vanillin, and coumarin in imitation vanilla be continued.

REPORT ON MEAT AND MEAT PRODUCTS

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.), *Referee*

Further collaborative work was done on the method for the determination of nitrates in meat and meat food products that was studied last year. A set of standard samples was prepared and sent to several collaborators. The results reported were not wholly satisfactory, apparently because of changes taking place in the samples. It is the opinion of the Referee that the method is not at fault, but that since the samples were held under differing conditions and for varying periods prior to analysis, the results are not strictly comparable. However, it is believed advisable to have more conclusive evidence on this point before the method is recommended for adoption. It is recommended[1] that further study be made during the coming year.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 21, 70 (1938).

REPORT ON SPICES

A METHOD FOR THE EVALUATION OF MARJORAM

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

The method submitted to the collaborators was published in *This Journal,* 17, 70 (1934).

To determine the practicability of this method, the whole leaves of a sample of marjoram were submitted to O. C. Kenworthy of this Station and B. J. Thiegs of the North Dakota Regulatory Department. The results of assay are shown in the following table:

	CLEVENGER	KENWORTHY	THIEGS
Volatile Oil (cc. per 100 g.)	1.66	1.68	1.75
Specific Gravity (25°/25°)	0.928	0.920	0.928
Optical Rotation*	6.8*	6.7*	—
Refractive Index (20° C.)	1.493	1.492	1.496
Acid Number	2.7	3.3	3.9
Ester Number	18.4	15.6	12.6

* Angular degrees 25° C. 100 mm. tube, white light.

The reasonable agreement in the yield and constants of the volatile oil of marjoram leaves obtained show that the present tentative method is applicable to marjoram leaves.

It is recommended[1] that work be continued on other spices.

No report on baking powders and baking chemicals was given by the referee.

REPORT ON FLUORINE IN BAKING POWDERS AND BAKING CHEMICALS

By DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

As the first step in the work on this type of products the Associate Referee chose the determination of fluorine in monocalcium phosphate.

Collaborative work was undertaken immediately by two methods:

(1) The peroxidized titanium method as used in previous years for the collaborative work on fluorine in foods, *This Journal,* 16, 612 (1933).

(2) A modification of the thorium nitrate titration method suggested by W. S. Allen and presented elsewhere in this issue (p. 459).

[1] For report of Subcommittee C and action by the Association, see *This Journal,* 21, 71 (1938).

DISTILLATION (common to both methods)

Transfer 20 grams of sample to a 125 or 250 cc. Claissen flask, and add 10 cc. of water and 20 cc. of 60% $HClO_4$. Distil according to the Willard and Winter procedure,[1] collecting 250 cc. at 135° C. ±3°. Make alkaline with 0.1 N NaOH, adding 0.2 cc. in excess. Evaporate in porcelain to 5 cc. volume, transfer to a 125 cc. Claissen flask, add 12 cc. of H_2SO_4, and again distil at 135° C. ±3°. Collect about 200 cc. of distillate (150 cc. should be collected above 132° C.). Make to 200 cc.

NOTE: Before each distillation is made the Claissen flask and the pebbles should be thoroughly rinsed with *boiling* 10% NaOH in order to eliminate all traces of gelatinous silica.

COLLABORATORS

The following collaborators reported: Howard Adler, Victor Chemical Works; W. S. Allen, General Chemical Co.; R. W. Bridges, Aluminum Company of America; Jonas Carol, U. S. Food and Drug Administration; J. N. Carothers, Monsanto Chemical Co.; J. R. Davies, General Foods Corp.; W. K. Enos, Virginia-Carolina Chemical Corp.; J. B. Fullerton,

TABLE 1.—*Results of collaborative study**

ANALYST NO.	PEROXIDIZED TITANIUM		TITRATION		REMARKS
	AVERAGE	VARIATION	AVERAGE	VARIATION	
1			8.3	8.0 - 8.5	Own method: 8.5
2	3.4	3.2- 3.5	4.3	4.25- 4.38	
3	5.1	4.6- 5.5			
4	8.3		6.5		
5	8.0		6.6		
6	6.3	5.0- 7.0	10.8	5.0 -14.0	
7	9.4	8.8-10.0	8.2	6.6 -10.3	Own method: 6.5
8			6.7	6.6 - 6.7	
9	5.7	5.0- 6.3	7.7	7.5 - 7.8	Own method: 6.7
10	5.5	5.0- 6.0	5.5		
11			7.6	7.4 - 8.0	Own method: 8.2
12			7.5	7.0 - 8.0	Own method: 6.0
13			7.3		Own method: 7.2
14	7.6	5.3- 9.5	10.2	5.8 -14.5	Own method: 8.3
15	8.5	7.5-10.0	6.8	6.0 - 7.4	
16	6.7	4.0- 7.3	9.8	9.0 -11.9	
17	5.2	4.0- 6.0	6.9		Own method: 4.5
18			6.7	6.6 - 6.9	
19	7.9	7.5- 8.4	7.5	7.2 - 8.0	
20	6.3				
21	6.3	6.3- 6.4	7.6	7.1 - 8.0	
22	9.9	8.0-11.8	13.2	11.8 -14.6	
23	13.7	12.0-15.0			

* Results are listed in the order the reports were received by the Associate Referee. The average and standard deviation, respectively, are for the

Peroxidized titanium method: 7.28±2.39
Thorium nitrate method: 7.79±1.97

[1] *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1933).

The Upjohn Co.; M. Given, Eastman Gelatine Corp.; V. L. Harnack, United Chemical and Organic Products; Arthur D. Holmes, The E. L. Patch Co.; F. Visser't Hooft, Lucidol Corp.; George E. Keppel, U. S. Food and Drug Administration; Simon Klosky, The American Agricultural Chemical Co.; H. V. Moss, Monsanto Chemical Co.; W. C. Motz, Virginia Chemical Corp.; K. B. Peterson, North Dakota Regulatory Dept.; G. D. Richards, Jaques Manufacturing Co.; W. E. Stokes, Standard Brands, Inc.; O. I. Struve, Eastern States Coop. Milling Corp.; F. O. Taylor, Parke, Davis & Co.; Harley G. Underwood, U. S. Food and Drug Administration; and the Associate Referee.

Table 1 gives the results by the different methods as well as high and low results for collaborators reporting more than one analysis.

The thorium nitrate titration method seems to give slightly higher results. In part at least, this is no doubt due to the use of sulfuric acid in the second distillation. Reports from several of the collaborators in regard to the acidity of the distillates indicate amounts varying from 1 to 11 mg. of sulfuric acid per 200 cc. of distillate and from 30 to 1500 mg. of perchloric acid per 250 cc. of distillate.

Even traces of sulfuric acid cause an increase in the consumption of thorium nitrate during the titration. Experiments made by the Associate Referee indicate that 0.2 mg. of sulfuric acid per 40 cc. in the absence of fluorine may cause a blank, corresponding to about 0.005 mg. of fluorine. With the proportions used in the analysis of this sample it seems possible, therefore, that a plus error of 1 p.p.m. or more could be ascribed to the use of sulfuric acid. In the future the use of 20 cc. of perchloric acid in the second distillation will be recommended.

It is recommended that the work on fluorine in baking powders and baking chemicals be continued.

REPORT ON LEAD IN BAKING POWDERS AND BAKING CHEMICALS

By P. A. Clifford (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The general methods for lead appear to be easily adaptable to the determination of lead in baking powders and baking chemicals. Simple solution of the sample in acid or water, as outlined in *Methods of Analysis, A.O.A.C.*, 1935, p. 389, **27**, can be employed in the case of most baking powder chemicals; the sulfide method of separation can be used for phosphates and possibly sulfates, and the dithizone method of isolation for others. The analysis is complicated, in the case of baking powder itself, by the presence of starch.

The only practical method of sample preparation (in the case of baking

powders) appears to be an ashing procedure. To test this procedure, varying amounts of lead were added to 25 gram samples of a combination baking powder testing low in lead, and the samples were placed directly in the muffle at 500° and ashed. Low results, ranging from 80 to 90 per cent, were obtained.

The source of these lead losses was investigated, and they appeared to be due solely to high temperatures developed during the ashing. The baking powder ashed very rapidly, and apparently local overheating developed during the process. Recoveries of 92.5 per cent were obtained when the ashing was conducted at 475°, and of 95.5 per cent when the ashing temperature was 450°. Theoretical recoveries, even at 500°, were obtained when the baking powder was first charred black upon the hot plate before being placed in the muffle. Satisfactory results were also obtained when the baking powder was given a preliminary charring in the muffle at 300° for 30 minutes, and the ashing finished at 500°. Apparently, when the sample of baking powder is placed directly in the muffle at 500°, the loss of lead takes place in the first stages of combustion. This lead loss during the preliminary stages of the ashing may be a general difficulty with food materials high in carbohydrates. It is probably due to too rapid combusion and local overheating at the surface of the sample, but the possibility of lead loss due to volatilization of the lead in combination with acidic constituents liberated as a result of the breaking down of carbohydrates is also to be considered. Lead losses during the ashing of other carbohydrate foods, particularly maple sirup and sugar, have been reported recently. The entire question appears to need attention. If the work on baking powders reported here is any indication, the situation may be saved by a careful preliminary charring of carbohydrate foods, either on the hot plate or in a temperature-controlled muffle at not over 300° until all volatile organic matter is driven off and a dry char results. Of particular interest is the "radiant heater" devised by Nims and Horwitt[1] and recently recommended by Horwitt and Cogwill[2] for the preliminary charring of lead samples before ashing. With this device, moist samples can be dried (and charred), and dry samples can be charred to any desired degree, before ashing, by the application of overhead heat developed by means of an electrical heating unit. The precaution of covering the floor of the muffle with an asbestos board should *always* be observed.

With specific reference, once more to baking powders, the following procedure is suggested. The Referee expects to submit it, with possible slight modifications, to collaborative study during the coming year:

Place 25 grams of baking powder (10 grams may be used if the more sensitive colorimetric dithizone procedure is to be used in the final estimation) in a 4–5 inch

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[1] *J. Ind. Eng. Chem. Anal. Ed.*, **8**, 275 (1936).
[2] *J. Biol. Chem.*, **119**, 553 (1937).

casserole and char on the hot plate or by means of an overhead "radiant heater" (see above reference) until a crisp, dry char is obtained. Place the casserole in the muffle at 450° and ash (approx. 1 hour) until the bulk of carbonaceous material is consumed. Withdraw the casserole, allow to cool, and wet the char with about 10 cc. of concentrated HNO_3, added dropwise. Break up lumps, if necessary, with a flattened stirring rod, rinse down, dry, and replace in the muffle for 15 minutes. (A perfectly carbon-free ash should be obtained.) Cool, add 30 cc. of concentrated HCl, cover with a watch-glass, and heat gently on the hot-plate or steam bath until the ash is disintegrated, then bring nearly to dryness on the steam bath. Add another portion of HCl, 20 cc., warm, and add hot water (150–200 cc.) until solution is complete. (Very little insoluble material should remain.) Flush the solution, and insoluble residue, if any, into a 500 cc. Erlenmeyer flask, add 10 grams of citric acid, or preferably its equivalent, in lead-free NH_4 citrate, and bring the solution to about *p*H 3.5 with ammonia (bromophenol blue). Add a few mg. of copper salt, cool, and pass in H_2S for about 5 minutes. Filter immediately on a fritted glass filter, leaving as little as possible of the sulfides in the flask. From this point proceed as directed in paragraph 17(a), p. 382 beginning—"If there is a possibility of the sulfide precipitate being contaminated with Cl, As_2O_3, P_2O_5 etc." Determine the Pb as directed in 19 or 21.

RECOMMENDATIONS[1]

It is recommended—

(1) That work on the determination of lead in baking powders and baking powder chemicals be continued.

(2) That collaborative work be undertaken to test the applicability of the general lead methods to this problem.

No report on nuts and nut products was given by the referee.

REPORT ON FISH AND OTHER MARINE PRODUCTS

By H. D. Grigsby (U. S. Food and Drug Administration, Philadelphia, Pa.), *Referee*

In accordance with recommendations approved last year, the Referee studied the methods compiled by P. B. Clarke and published with the last report, *This Journal*, **20**, 410 (1937).

A letter containing these methods with some modifications and extensions was circulated to collaborating chemists, with request for critical comment. Very few suggestions for changes in the methods were proposed.

Methods for preparation of samples are based on the experience of analysts working with the products in question and similar products. Other methods proposed are mainly those already official in Chapter **XXVIII**, Meat and Meat Products, *Methods of Analysis, A.O.A.C.*, 1935.

No collaborative analyses were requested.

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 21, 71, 75 (1938).

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods proposed for preparation of sample and for the determination of ash, salt and total nitrogen be adopted as tentative. These methods were published in *This Journal*, **21**, 85 (1938).

(2) That further study be made of methods for the determination of total solids and ether extract.

REPORT ON CACAO PRODUCTS

By W. O. Winkler (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The work this year was confined mainly to the detection of shell, although a little work was done on milk solids, and extra samples were prepared in anticipation of work on lecithin.

In preparation for the work on cacao shell, authentic samples of sweet chocolate and sweet milk coating were prepared at a chocolate factory. Due to the rush of other work, this was not done in time to send out collaborative samples. However, the Referee was able to secure the help of one collaborator for this work.

The method for the determination of pectic acid in bitter and sweet chocolate, reported last year, was used, with a few slight changes in the procedure. The precipitation with tannic acid is now done at 8°–10° C. rather than at room temperature. The residue, after extraction of the pectin, is dried and weighed, and the fat-free cacao mass is obtained by the use of a factor. Further changes are the deletion of the calcium precipitation and the introduction of an alcohol extraction following the ether extraction. Weights of samples taken have been increased, and the volume of extraction solution has also been increased from 210 to 350 cc. The new samples obtained this year show even a greater difference between shell and nibs in pectic acid content than was found previously. The values obtained were 0.10–0.20 per cent for nibs and 4.8 per cent for shell on the basis of the fat-free mass.

Table 1.—*Collaborative results on pectic acid, on fat-free, sugar-free basis (per cent)*

	SAMPLE 1* NO ADDED SHELL	SAMPLE 2* 9.45% ADDED SHELL	SAMPLE 3 100% SHELL
J. B. Wilson	0.08, 0.16	0.60–0.62	
W. O. Winkler	0.12, 0.20	0.67–0.71	4.8

* Original liquor, less than 0.5% shell, fat-free basis.

A sample of sweet chocolate containing 9.45 per cent of added shell on the basis of the fat-free, sugar-free cacao mass, was prepared. The original

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **21**, 70 (1938).

sweet coating and the one containing the added shell were analyzed by two collaborators for pectic acid. The results are given in Table 1.

The results indicate that the method should give a good estimate of the shell content of a sample; they are almost identical with those calculated from the shell present.

Content of pectic acid may also be used in connection with the crude fiber figure. A multiplication of the two figures gives an increased ratio between shell and nibs. The Referee determined fiber on the samples given in Table 1, multiplied the figures, and obtained the figure 75 for the shell and 1 for the nibs. A spread of this magnitude should offer increased ability in diagnosing presence of shell.

No method for determining pectic acid in milk chocolate was reported last year, but the following procedure was worked out this year.

PECTIC ACID IN MILK CHOCOLATE

Place 60 grams of milk chocolate in a centrifuge bottle (250 cc. or larger). Extract the sample with two 125 cc. portions of ether. Shake the bottle each time until the mixture is uniform and centrifuge for 7 or 8 minutes at about 1800 r.p.m. Decant the ether extracts. Place the bottle in a warm place to expel the ether. To use the vacuum to assist the vaporization, place a stopper in the bottle holding two glass tubes, the one open and the other attached to the vacuum. Draw a gentle current of air through the bottle.

Add to the dry residue in the bottle about 15 cc. of 0.5% ammonium oxalate solution. Mix the residue with the liquid with the aid of a stirring rod with a flat end, then add 155 cc. more of the oxalate solution, stopper, and shake until the mixture is quite uniform. Loosen the stopper and warm the solution to 45°–50° C. for 15 minutes with frequent mixing. Centrifuge for 15 minutes at 1800–2000 r.p.m., and decant the supernatant liquid into a 400 cc. beaker and reserve for further treatment.

Shake the stoppered bottle with 75 cc. of the oxalate solution and carefully decant it into a 500 cc. wide-mouthed Erlenmeyer flask without loss. Shake the bottle with additional portions of oxalate solution to remove all the residue to the Erlenmeyer. Add sufficient of the 0.5% oxalate solution to the flask to make a total of 350 cc. Cover the flask and place it on the steam bath for 3–3.5 hours with occasional shaking.

To the liquid in the beaker reserved above, add 90 cc. of water and add 1 cc. of glacial acetic acid per 100 cc. total volume. Allow the precipitate to settle and filter on a 11 cm. Buchner funnel containing a C. S. & S. No. 589 white ribbon paper overlain with paper pulp or filter cell. Wash the precipitate and beaker with a small amount of the oxalate solution containing 1 cc. of glacial acetic acid per 100 cc. Place the filtrate in a large beaker on the steam bath.

Remove the Erlenmeyer from the bath after sufficient time and transfer the contents to a centrifuge bottle. If the volume is too large, centrifuge in portions, adding the second portion to the residue in the bottle. Wash the Erlenmeyer with some water and a policeman and add to the bottle. Centrifuge each portion for 15 minutes at 1800-2000 r.p.m. Decant the liquid extract to a beaker, precipitate the protein with acetic acid, and filter as with the former extract. Transfer the residue in the centrifuge bottle to a tared dish containing some asbestos, dry at 100° C., and weigh. Multiply the weight by the factor 1.90 to obtain the fat-free cacao mass in the sample. Combine the extracts in a larger beaker (at least 1000 cc.) and

evaporate to about 125–150 cc. on the hot plate. Transfer the liquid to a centrifuge bottle and proceed with the tannic acid precipitation and determination of pectic acid as directed in the method for bitter and sweet chocolate published last year, *This Journal*, 20, 417 (1938). Calculate the pectic acid from the fat-free milk and sugar-free cacao found.

Determinations on the sample of milk chocolate prepared by the method gave about the same result as was obtained on the sweet chocolate. No collaborative work was done on milk chocolate samples.

LECITHIN

No work was done on methods for lecithin during the year, although a number of methods were reviewed by the Referee. No appointment of an associate referee was made. It seems imperative that work be done, not only on lecithin but on a variety of products on the market advocated for uses similar to lecithin. These products are largely synthetic and are mainly phosphorized and sulfonated fats.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be done on the method for the determination of pectic acid in chocolate as an index of shell content.

(2) That methods for the determination of lecithin and other products used for similar purposes be studied.

No report on gums in foods was given by the referee.

REPORT ON OILS, FATS, AND WAXES

By G. S. Jamieson (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

During the past year a collaborative study was made of several methods for the determination of free fatty acids in both crude and refined oils under the direction of Associate Referee R. S. McKinney, who will present a report to the Association with recommendations.

Owing to unavoidable circumstances, Associate Referee Lawrence Zeleny was not able to arrange for a collaborative study on the method for the refractometric determination of oil in seeds.

At the suggestion of the Referee, McKinney also took charge of the collaborative study authorized by the Association on methods for the determination of the thiocyanogen value of fats and oils. The report on the results of this investigation will be presented by him.

The Referee offers the following recommendations[2]:

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 21, 75 (1938).
[2] For report of Subcommittee C and action by the Association, see *This Journal*, 21, 71 (1938).

(1) That the Fitelson method for the detection and estimation of tea seed oil be made official (final action).

(2) That the Kaufmann method for the determination of the thiocyanogen value of fats and oils be made official (first action).

(3) That the methods of the National Cottonseed Products Association for the determination of free fatty acids in crude and refined oils, *This Journal,* **21**, 88 (1938), be made official (first action).

(4) That a collaborative study be made on the application of the refractometric method to the determination of the oil content of one or more commercially important oil seeds under the direction of Associate Referee Zeleny.

(5) That studies on acetyl and hydroxyl values be discontinued for the present because of their limited application and because of the press of work on studies of methods of more general application.

NEW WORK

It is suggested that Associate Referee McKinney be authorized to undertake a collaborative study on the determination of the Polenske number. Several investigators have called attention to the different results obtained for a given sample of oil by using pumice of different sizes with the distillation of the volatile acids. Powdered pumice gave the highest values. With the Reichert-Meissl determination, the size of the pumice used appeared to have little effect upon the results obtained.

During the past year, McKinney made a series of experiments with several kinds of palm kernel oils, using powdered pumice (60–80 mesh) and fragments as directed by the Association's method. In each case the use of powdered pumice gave Polenske numbers about one unit higher than those obtained by the official method.

In practice, the present situation is that there are many following directions that specify the use of powdered pumice, whereas others follow the Association's method.

No report on refractometric determination of oil in seeds was given by the associate referee.

REPORT ON THE THIOCYANOGEN NUMBER OF FATS AND OILS

By R. S. McKinney (Carbohydrate Research Division, Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In accordance with the recommendations adopted by the Association, a collaborative study was made of the F. A. C.[1] and the modified Kaufmann[2] thiocyanogen methods.

[1] *Ind. Eng. Chem. Anal. Ed.*, 8, 233 (1936).
[2] Jamieson, G. S., "Vegetable Fats and Oils," American Chemical Society Monograph Series, New York, 1932.

Two samples of refined oil were submitted to the following seven collaborators.

COLLABORATORS

1. L. M. Tolman, Wilson and Company, Inc., Chicago, Ill.
2. R. C. Newton, Swift and Company, Chicago, Ill.
3. R. S. McKinney.
4. J. T. R. Andrews, The Proctor and Gamble Company, Ivorydale, Ohio.
5. H. J. Alleman, The Kroger Food Foundation, Cincinnati, Ohio.
6. The Barrow-Agee Laboratories, Memphis, Tennessee.
7. R. T. Munsberg, Edgewater Control Laboratory, Lever Brothers Company, Edgewater, N. J.

Sample No. 1 was a refined corn oil and Sample No. 2 was a refined cottonseed oil. Table 1 gives the results obtained for the thiocyanogen

TABLE 1.—*Thiocyanogen numbers by Methods I and II*

COLLABORATOR	METHOD I. F. A. C.		METHOD II. KAUFMANN	
	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2
1	80.1 81.8	72.3 72.8	75.5 75.6	67.1 67.0
2	69.2 69.0 } out	62.6 62.7	77.0 76.4 77.0	69.4 69.0 69.1
3	77.9 77.8 77.9	68.4 68.3 68.6	78.0 77.5 77.6	69.0 68.8 68.8
4	80.7 81.6 80.8	72.1 72.1 71.6 71.9	81.3 81.3 } out	72.2 72.5 } out
5	80.2 80.3 80.5 82.9	72.1 72.1 71.6 73.1	76.2 74.1 77.0 78.1	69.5 68.6 69.0 68.3
6	80.5 78.9 79.0	69.5 70.1 70.6	76.7 76.3 76.9	68.3 68.4 68.3
7	77.2 77.2 77.6	68.0 68.3 68.5	76.2 76.2 76.3	68.1 68.1 68.0
Av.	(1) 78.1 (2) 79.3	(1) 69.5	(1) 77.2 (2) 76.6	(1) 69.0 (2) 68.4
Av. Variation	2.9 1.5	2.6	1.3 0.5	1.1 0.6
Max. Variation	9.0 2.0	6.8	3.9 1.1	3.3 1.3

number of these oils by Method I (F. A. C.) and Table 2 gives the results obtained on these oils by Method II (Kaufmann).

COMMENTS AND CONCLUSIONS OF THE ASSOCIATE REFEREE

It was found that the value obtained by Collaborator 2 by Method I on Sample 1 was outside the accepted variation from the average value and that the values obtained by Collaborator 4 by Method II on Samples 1 and 2 were likewise outside the accepted variation from the average values. Therefore, these values were eliminated before the reported averages were calculated.

Sample 1, a refined corn oil containing 10.33 per cent saturated acids (Bertram) and with an iodine value of 126.35 (Hanus), was calculated to have a thiocyanogen number of 77.4. The average thiocyanogen number obtained on this oil by Method I (F. A. C.) is 79.3 and by Method II (Kaufmann) it is 76.6.

Sample 2, a refined cottonseed oil containing 19.66 per cent saturated acids and with an iodine value of 109.30, was calculated to have a thiocyanogen number of 68.6. The average thiocyanogen number obtained on this oil by Method I is 69.5 and by Method II it is 68.4.

A comparison of the thiocyanogen numbers obtained by the collaborators on these oils shows that Method II (Kaufmann) not only gave results that are closer to the theoretical, but they also are much better both in regard to the average variation and the maximum variation from the accepted average value.

After this work was started the Associate Referee found that it was necessary to use 100–150 per cent excess of the reagent in determining the thiocyanogen number of an oil. When a larger excess was used, the values were about three points higher than the calculated or theoretical values.

One collaborator suggested that a pipet be substituted for the buret used in Method II for measuring out the thiocyanogen solution to prevent undue exposure of the solution to the air. The Associate Referee concurs with this suggestion.

It is recommended[1] that the Kaufmann method for determining the thiocyanogen number of fats and oils, as modified by the Associate Referee, be made tentative. The method was published in *This Journal*, **21**, 87 (1938).

REPORT ON FREE FATTY ACIDS

By R. S. McKinney (Carbohydrate Research Division, Bureau of Chemistry and Soils, Washington, D.C.), *Associate Referee*

In accordance with the recommendations adopted by the Association, a collaborative study was made of the N. C. P. A. method[2] for determin-

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 21, 71 (1938).
[2] Methods of Chemical Analyses of the National Cottonseed Products Association, Memphis, Tenn.

TABLE 1.—*Collaborative results on Samples 1 and 2*

COLLABORATOR	METHOD I (A. O. A. C.)	METHOD II (N. C. P. A.)	METHOD III (COLD TIT.)
	Sample 1		
1	0.023	0.025	0.025
2	0.039	0.029	0.035
	0.039	0.029	0.035
3	0.061	0.027	0.027
		0.023	0.027
4	0.032	0.030	0.031
	0.032	0.030	0.031
5	0.034	0.028	0.035
	0.031	0.029	0.034
6	0.055	0.035	0.034
Av.	0.040	0.029	0.031
Av. Variation	0.010	0.003	0.004
Max. Variation	0.021	0.006	0.006
	Sample 2		
1	0.030	0.030	0.030
2	0.046	0.040	0.043
	0.046	0.040	0.043
3	0.051	0.038	0.037
	0.049	0.037	0.035
4	0.039	0.040	0.041
	0.039	0.040	0.041
5	0.034	0.042	0.045
	0.031	0.044	0.044
6	{0.052	{0.038	{0.037
	0.042	0.038	0.039
Av. Variation	0.008	0.003	0.005
Max. Variation	0.012	0.008	0.009

ing free fatty acid of crude oils, and of the N. C. P. A. method,[1] the present A. O. A. C. method,[2] and a cold titration method for determining the free fatty acids of refined oils.

Two samples of crude oil and two samples of refined oil were submitted to the following six collaborators:

[1] Methods of Chemical Analyses of the National Cottonseed Products Association, Memphis, Tenn.
[2] *Methods of Analyses, A.O.A.C.*, 1935, p. 417.

COLLABORATORS

1. L. M. Tolman, Wilson and Company, Inc., Chicago, Ill.
2. R. C. Newton, Swift and Company, Chicago, Ill.
3. R. S. McKinney.
4. J. T. R. Andrews, The Proctor and Gamble Company, Ivorydale, Ohio.
5. H. J. Alleman, The Kroger Food Foundation, Cincinnati, Ohio.
6. The Barrow-Agee Laboratories, Memphis, Tenn.

Samples 1, 2, 3, and 4 consisted of refined corn oil, refined cottonseed oil, crude soybean oil, and crude corn oil, respectively. Table 1 gives the results obtained for the free fatty acid content of refined oils Nos. 1 and 2 by the A. O. A. C., the N. C. P. A., and the cold titration methods.

Table 2 gives the results obtained for the free fatty acid content of the crude oils samples, Nos. 3 and 4, by the N. C. P. A. method.

TABLE 2.—*Collaborative results on crude oils.*

COLLABORATOR	SAMPLE 3	SAMPLE 4
1	2.38	5.32
2	2.35	5.35 5.35
3	2.44	5.40
4	2.25 2.23	5.38 5.34
5	2.31 2.33 2.33 2.33	5.36 5.31 5.36 5.34
6	2.3	5.3
Av.	2.35	5 35
Av. Variation	0.05	0.02

The N. C. P. A. method for the free fatty acid content of crude oils gave satisfactory results, and it is recommended[1] that this method be made official (first action).

The N. C. P. A. method also gave the best results for the free fatty acid content of refined oils, and it is recommended that this method be made official (first action).

These methods were published in *This Journal*, 21, 88 (1938).

No formal report on microchemical methods was given by the referee.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 21, 71 (1938).

REPORT ON MICROBIOLOGICAL METHODS

By ALBERT C. HUNTER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

In previous years proposed methods for the microbiological examination of sugar and canned meats have been submitted and have been published, *This Journal*, **19**, 439 (1936) and **20**, 429 (1937). The program outlined last year contemplated the formulation of methods for non-acid canned vegetables, canned tomato products, and fruits, and canned fish, to be presented at this meeting in order that they might be set up for further collaborative study. This plan is being carried out, and the associate referees on the respective products have drawn up proposed methods for canned vegetables, canned tomatoes and fruits, and canned fishery products. In these methods the associate referees have incorporated the basic procedures reported upon in the programs in past years with such minor modifications as are deemed necessary for each specific product. The procedures outlined in these methods are drawn from a background of experimental work, as well as from the broad experience of the associate referees and those with whom they have consulted.

The methods for sugar and canned meats previously proposed are being subjected to further study by the associate referees and their collaborators. There is no change to suggest in the method for canned meats at this time and there will be no report by the associate referee on that commodity. At this time no recommendation for the tentative adoption of either the method for sugar or for canned meats is being made, as it is considered advisable to leave the proposed methods open for further study of their general applicability during the time when the methods proposed at this meeting are also being subjected to collaborative study.

The work on microbiological methods in this Association has been moving along slowly now for a period of four or five years. It has taken that long for those who have been participating to become oriented and to settle into an organized program. At the beginning it was thought desirable to restrict all efforts to a fairly narrow field until there seemed to be prospects of making headway in a program viewed with skepticism by many bacteriologists. Now that methods have been proposed for four large classes of canned foods, and for sugar, it seems that another step might be taken in the expansion of the program and attention given to methods for the bacteriological examination of eggs and egg products. The recommendations[1] of the Referee are as follows:

(1) Reappointment of the present Associate Referees on (a) Canned Vegetables, (b) Canned Tomatoes and Fruits, (c) Canned Fishery Products, (d) Canned Meats, and (e) Sugar.

(2) Appointment of an Associate Referee on the Bacteriological Examination of Eggs and Egg Products.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **21**, 72 (1938).

REPORT ON MICROBIOLOGICAL METHODS FOR THE EXAMINATION OF CANNED FISHERY PRODUCTS

By O. W. LANG (The George Williams Hooper Foundation, San Francisco, Calif.), *Associate Referee*

The different types of canned fishery products that are marketed may be characterized by the method employed in their preservation. The common methods used are: (1) boiling water bath or steam under pressure in a properly equipped retort; (2) acidulation in vinegar; and (3) salt curing. Products canned under the second classification, *i.e.*, pickled herring, depend chiefly on acid concentrations ranging from *p*H 3.8 to 4.6 and moderately cool storage conditions for preservation. Heavily salt cured products such as anchovies or cod fish, either in bulk or canned, depend solely on salt concentrations of 18 to 24 per cent and moderately cool temperature storage conditions as the preserving agents. Spoilage occurring in products packed under Method 2 or 3 may be attributed to low acid concentration (high *p*H), low salt concentration, or in both cases high storage temperatures. In addition to the cultural methods, which will be discussed later, it is necessary to determine the *p*H range in the acidified products and the salt concentration in products of the third classification. A disintegrated and liquefied appearance without pungent or putrid odors in either class 2 or 3 indicates autolytic breakdown brought about by high temperature storage.

The suggested bacteriological methods concern chiefly the examination of so-called "non-acid," "low-salt" canned fishery products. Such products may be examined for sterility (keeping qualities) or for causes of spoilage due to inadequate retort process or mechanical imperfections of the container. Sample cans that are submitted for sterility are in the main normal in appearance. Those cans submitted for determination of spoilage causes are usually abnormal to a degree that characterizes them as "flippers," "springers," "soft swells," or "hard swells." These cans may be further defective in that they have certain mechanical imperfections such as dents due to rough handling, faulty cover or side seams, or perforations.

PRELIMINARY INCUBATION

All normal cans submitted for sterility tests and examination for keeping quality should be incubated at 37.5° C. for a period of one month prior to cultural examination. Cans should be laid on their sides in the incubator to prevent drying of the can seams. It is well also to move the container slightly at intervals during the course of incubation. A record should be made of any changes occurring in the condition of the container during incubation.

Although an incubation period of one month is proposed here the time of incubation adopted and reported in the literature by other investi-

gators has been found to vary. Weinzirl[1] used 7 days or longer at 37.5° C.; Savage, Hunwicke, and Calder[2] used incubation periods of "several weeks," and Cheyney[3] records 1–6 weeks, with a 2 weeks' period as the general average. Cameron, in his report on "Methods for the Examination of Canned Meats," *This Journal*, **20**, 429 (1937) states:

> . . . unspoiled samples submitted for examination as to keeping quality should be incubated at 37.5° C. for a period of one month. This time of incubation, necessarily an arbitrary matter, is considered the longest period that is practicable in the usual case. Anaerobes, which at times may remain dormant for many months, may escape detection, but usually the likelihood of spoilage in the product under ordinary commercial conditions of handling is indicated in this time. It is regarded as unnecessary to incubate at thermophilic temperature, *i.e.*, 55° C., because such spoilage in meat products is extremely rare.

The same conditions cited by Cameron prevail with respect to non-acid canned fishery products, and with such products, as with meat products, it is unnecessary to test for thermophilic bacteria by incubating at 55° C. according to Lang.[4]

A. Physical Examination and Preparation of the Can.

1. Make a complete set of records concerning the cans submitted (origin of samples, type of product, code marks, label, pertinent information concerning raw material and its pretreatment, and retort processes).

2. Make a record of the condition of the samples, noting mechanical defects, perforations, rust spots, dents, and all other can abnormalities. When spoilage is involved, ascertain the extent and determine whether one or several days' packs are affected.

3. Take all possible precautions to provide aseptic conditions when the cans are opened for cultural or enrichment examination. Have available a clean and disinfected table or desk top constructed of some type of impervious material. (If airborne contamination is imminent, it is advisable to carry out the cultural or enrichment procedure in a previously disinfected room, or under a hood especially designed for this purpose.) Scrub the container with sand soap, using a brush and warm water. To remove grease, oil, or outside lacquer, wipe the can with some suitable solvent (a solution of equal parts of ethyl alcohol and ethyl ether is recommended).

4. Sterilize the surface of the can at the point to be opened by holding and slowly rotating the can inverted in the flame of a Bunsen burner. (Rotating will serve to secure an even area of sterilization and will prevent scorching the contents.) Heat flat or normal cans in this manner until one of the ends becomes distended, since internal pressure promotes an expelling action and helps to avoid contamination when the can is opened. When examining swollen cans do the flaming with caution, and if necessary reduce the internal pressure by refrigeration of the container prior to flaming. When flaming is not practicable, follow the procedure involving treatment with bichloride of mercury (1+1000) described by Fellers, *This Journal*, **19**, 430 (1936), or that suggested by Cameron, *Ibid.*, **20**, 431 (1937), calling for thorough cleansing with 60% alcohol.

B. Removal of Sample.

1. Open the can at the point of sterilization with a suitable and previously

[1] *J. Med Res*, 39, 349 (1919).
[2] Special Rept. No. 11, Food Investigation Board, London, England (1920).
[3] *J. Med. Res*, 40, 177 (1919).
[4] Univ. of California Publications in Public Health, Vol. 2, No. 1 (1935).

sterilized opener. Have the opening as close to the center of the cover as possible and only large enough to permit adequate sampling. (A long handled can opener designed to cut in the circular direction in which the cutting edge travels as the circumference of a circle is best adapted for this purpose. This type of can opener lends itself quite readily to individual wrapping and sterilization, and also has the added advantage of preventing contact of the analyst's hands with the sterilized portion of the can.)

2. Determine the type of instrument to be used for removing the inoculum by the nature of the product under examination. (This operation is discussed by Tanner, *This Journal,* 19, 432 (1936).) For consistently satisfactory results for canned fishery products use either large aperture pipets or sterile teaspoons, depending upon the product. Sample liquid or semi-liquid products with large-bore, untapered sterile pipets. Sample solid materials with teaspoons or other suitable instruments, such as cork borers or brass tubes that have been sterilized, *This Journal,* 20, 431 (1937). (One advantage in using teaspoons is that a separate spoon may be used for each can, the spoons having been previously sterilized by being placed in a large metal receptacle and subjected to 15 pounds' steam pressure for 60 minutes.) Remove from the product a representative sample of 10–15 grams of material for inoculation into culture media. In making the inoculations carefully observe that the ratio of the volume of the sample to that of the medium is not such as to affect the *p*H of the medium. If the can contents are fluid, tube and plate cultures may be made directly. If the material is solid, it may be inoculated directly into medium wide-mouthed flasks, which are cork stoppered and covered with vegetable parchment paper, or if plate cultures are desired, the solid material may be shaken in a sterile bottle with sterile broken glass and sterile saline solution until a proper suspension is secured for use as inoculum.

Place an additional sample in a sterile flask and hold in reserve under refrigeration for possible reexamination or for toxicity tests.

C. Culture Media.

Prepare both anaerobic and aerobic cultures from the material secured as a sample. In preparing anaerobic cultures, use an unheated portion of the sample and a portion that has been heated for 30 minutes at 80° C. to destroy vegetative forms in order to test for the presence of anaerobic spores. Prepare an aerobic broth culture. (The preparation of plate cultures is optional and may be desirable under some circumstances.)

The culture media suggested by Cameron, *This Journal,* 20, 432 (1937), are well adapted to the examination of fishery products. Those prepared from dehydrated media marketed by the Difco Laboratories. Inc., Detroit, Michigan, are especially suitable. Considerable success has been attained with the following media, which are also recommended:

Beef Heart Broth

Distilled water	1000 cc.
Minced fresh fat-free beef heart	500 grams
Difco Neopeptone	10 grams
C. P. NaCl	5 grams

With the exception of the NaCl, mix the ingredients in a steam kettle and boil for approximately 10 minutes. Remove the broth, filter, and adjust to *p*H 7.6–7.8. Boil gently for approximately 10 minutes, filter, and add the salt. Bottle or tube, preferably in hard glass containers, and sterilize for 20 minutes at 115° C. The range of the final *p*H should be from 7.0 to 7.4.

Beef Heart Agar

Add 2% of agar to the above broth; or preferably mix the shredded agar with 500 cc. of the distilled water required, using the other 500 cc. for the preparation of the infusion. Then mix the two portions.

Heart Meat Broth for Anaerobic Enrichment

Place the residue of the ground beef hearts approximately 1–1.5 inches deep, into wide-mouthed flasks or tubes. Add the beef heart broth to bring the supernatant liquid 1–1.5 inches above the meat, stratify with vaseline, and sterilize in the autoclave at 10 pounds' steam pressure for 20 minutes.

(It is recommended that all culture media be incubated for 24–48 hours before use.)

D. *Incubation and Culture Study.*

Incubate all cultures for at least 48 hours at 37.5° C., and if practicable incubate anaerobic cultures at least 72 hours.

Use the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists as a guide for the further study of microorganisms obtained in the cultural procedure described.

No report on microbiological methods for canned meats was given by the associate referee.

REPORT ON MICROBIOLOGICAL METHODS FOR THE EXAMINATION OF CANNED VEGETABLES

By E. J. Cameron (Research Laboratories, National Canners Association, Washington, D. C.), *Associate Referee*

This report is limited to suggested methods for non-acid and semi-acid canned vegetables. Strictly speaking, all vegetables and fruits are acid to some degree, but for convenience they are grouped as non-acid, semi-acid, and acid. The non-acid products, such as peas and corn, are in the range of *p*H 6.0–7.0. The semi-acid products, such as string beans, spinach, and asparagus, are commonly between *p*H 4.5 and 6.0, most of them higher than 5.0. The upper limit of the acid range may be taken at *p*H 4.5. This is about the point below which spore-forming bacteria cease to be factors of spoilage, and it becomes unnecessary to use a process sufficiently severe to destroy them.

Necessarily these groupings are more or less arbitrary, and while there are microbiological similarities within each group and some differences between the groups, there remains the fact that the same general classes of microorganisms are to be considered in the examination outlined here.

The procedure to be followed in the microbiological examination of canned vegetables is indicated by the purpose of the examination. Samples are commonly submitted to the laboratory for one of the following three purposes:

(1) Unspoiled samples—for direct bacteriological examination for sterility.

(2) Unspoiled samples—for examination as to keeping quality.

(3) Spoiled samples—for examination as to cause of spoilage.

The technic of examination for all three types of samples is similar with respect to the treatment of the container, the removal of the samples, and culture methods. Differences in treatment are those indicated in the Report on Methods for the Examination of Canned Meats, *This Journal,* **20,** 429 (1937), with the exception that with canned vegetables it is frequently necessary to incubate at 55° C. in order to determine the possibility of thermophilic spoilage in the event of undercooling following processing, or where it is desired to determine keeping qualities in tropical climate. At 55° C. an incubation period of 10 days is recommended.

The various steps in culturing technic are described in order as follows:

A. Physical Examination and Preparation of the Can.

Follow the procedure suggested in Report on Methods for the Examination of Canned Meats (*loc. cit.*).

B. Removal of Sample.

Follow procedure suggested under B1–2 in Report on Methods for the Examination of Canned Meats (*loc. cit.*).

C. Culture Media.

The Report on Culture Media for Non-acid Products, *This Journal,* 19, 434 (1936), referred to the various bacterial types concerned in understerilization of non-acid products and remarked upon the difference in flora when spoilage resulted from leakage. Five media, which are widely used in laboratories interested in canning problems, were recommended. These media were (1) dextrose tryptone agar for the detection of "flat sour" bacteria; (2) liver broth intended for the detection of thermophilic anaerobes, putrefactive anaerobes, and other mesophilic anaerobes; (3) beef heart peptic digest broth intended principally for the detection of putrefactive anaerobes and their cultural study; (4) corn liver medium intended for the detection of thermophilic anaerobes not producing hydrogen sulfide, putrefactive anaerobes, and other mesophilic anaerobes; and (5) sulfite agar intended for the detection of thermophilic anerobes producing hydrogen sulfide. In conjunction with a description of the media, incubation procedure was suggested, and diagnostic characteristics were described.

For routine culture purposes, dextrose tryptone agar and liver broth are suitable and their use is recommended. Where special examination is made for putrefactive anaerobes, beef heart peptic digest is recommended to supplement liver broth cultures. Corn-liver medium should supplement liver broth in special search for thermophilic anaerobes or in their study after isolation.

Sulfite agar is useful only when indicated by a type of spoilage characterized by the presence of hydrogen sulfide. Due to complaints charging that the preparation of the yeast water base used in this medium is too time-consuming for laboratories in which tests for sulfide spoilage organ-

isms were made infrequently another medium was devised which, in comparative tests, has been found to be superior to the original. The make-up of this medium follows, and it is now recommended as the principal medium for the detection of thermophilic anaerobes producing hydrogen sulfide: Water, 1 liter; tryptone, 10 grams; sodium sulfite, 1 gram; and agar, 20 grams. At the time of tubing, a clean iron strip or nail is placed in the tube. No adjustment in reaction is necessary. As in the original sulfite agar, the "sulfide" spoilage organisms are detected through the formation of characteristic blackened spherical areas. In the usual case there is no gas formation. The presence of gas coupled with the general blackening of the medium indicates the presence of thermophilic anaerobes not of the sulfide spoilage group. The darkening of the medium in this case results from the reduction of sodium sulfite by hydrogen gas.

REPORT ON CANNED TOMATO PRODUCTS

By B. A. LINDEN (Bacteriological Section, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In last year's report the subject of culture media for the bacteriological examination of acid canned food products was discussed, *This Journal*, **19**, 440 (1936). The additional work done this year on technical procedures and culture media showed sufficient promise to warrant their inclusion in a proposed method for the examination of tomato products and other acid canned foods.

This method has been tested to a limited extent in collaboration with various members of the staff of the Bacteriological Section of the Food and Drug Administration. Helpful suggestions were made by E. J. Cameron of the National Canners' Association Research Laboratories. It is now planned to enlist the aid of other collaborators in the field of food microbiology to ascertain the effectiveness of the proposed method.

There are three primary objectives sought in the microbiological examination of canned foods that come within the *p*H range of "acid products." They are—

(1) Detection of spoilage.

(2) Determination of commercial soundness (keeping quality).

(3) Determination of sterility and the detection of type of bacteria capable of causing spoilage when the product is utilized as a sauce with other non-acid type foods.

The procedure for all three objectives, with respect to treatment of container, removal of the sample, amount of inoculum, and cultural methods are essentially the same. Modifications in treatment include the following: (a) Before being cultured, samples submitted for examination for incipient or advanced spoilage should be examined microscopically in

order to obtain some information as to the types of organisms present. Gram's stain is useful as most spoilage organisms in this class of foods are Gram-positive. Determination of *p*H may be useful, but is of limited value as only slight reaction changes occur in these products. (b) Normal appearing cans submitted for examination for commercial soundness or keeping quality should be incubated at 30° C. if less than 14 days has elapsed since the product was packed. Additional incubation at this temperature to insure at least 14 days' incubation is desirable. When no 30° C. incubator is available, incubation at an average room temperature of 25° C. may suffice. (c) Samples examined for the presence of other than spoilage organisms require incubation at 37° C. in culture media suitable for the detection of organisms capable of causing spoilage of non-acid foods. Products such as tomato pastes, purées, and ketchups are often used as packing media (sauces) for non-acid products and may be responsible for spoilage under these changed conditions of *p*H. The following method is recommended.[1]

CULTURING PROCEDURE

A. PHYSICAL EXAMINATION AND PREPARATION OF CAN

1. Note and record all marks of identification, either embossed on the can or appearing on the label.

2. Remove the labels. Record any physical defects such as improper closure, leaks, bad seams, buckling, or other abnormal conditions such as springers, flippers, or swells.

3. Scrub the entire surface of the container with a brush, using soap and warm water, and wipe off the surface with 70% alcohol prior to opening.

4. If possible, open the end of the container that does not bear the cannery code marks. For sterilization at the site of opening, expose the container top over the flame of a Bunsen burner, using a rotary motion to obtain an even distribution of heat and to prevent scorching of contents. (This procedure also tends to minimize the danger of contamination from inrush of air when the container is opened.) Do not subject hard swells to heating before opening. (It is suggested that swollen containers that exhibit considerable pressure be chilled before they are opened to reduce spurting.) After cleansing swollen cans, apply the treatment described by Fellers, *This Journal,* **19**, 430 (1936). (As an alternative it is suggested that after the mechanical cleansing with soap and water the container be thoroughly cleaned with 70% alcohol. Phenolic solutions may interfere with the organoleptic examination of the can contents because they tend to mask the odors.)

B. REMOVAL OF SAMPLE

1. *Opening of container.*—After sterilizing the site of opening, make an entrance with an appropriate type of opener, which has also been sterilized by direct flaming. For products of heavy consistency, such as tomato paste, preferably use openers of the spiral or circular type, which cut around a central puncture. (Openers that cut around the can seam are less desirable because of greater surface exposure and danger of contamination.) When fluid products are sampled, make the opening by a suitable metal punch of appropriate diameter to insure an aperture that will

[1] For report of Subcommittee C and action of the Association, see *This Journal*, **21**, 72 (1938).

permit free access of the sampling pipet. Further details on sampling devices are discussed by Tanner in *This Journal*, **19**, 432 (1936).

2. *Inoculum.*—Sample liquid or semi-liquid food products with sterile untapered pipets or inverted tapered pipets of suitable capacity. (The untapered pipets should have minimum dimensions of 350 mm. in length and 5 mm. inside diameter.) Sample solid or semi-solid food products with sterile spatulas, long-handled spoons, or other instruments, as suggested by Tanner, depending on the character of the food under examination. Use pipets only for products of such viscosity as to permit transfer of the inoculum into the culture media by gravity. As suggested by Tanner, use a sample consisting of a minimum of 15 grams or 15 cc. of the food material and divide it into aliquots for duplicate culturing in each of the different culture media used in the examination.

3. *Sampling of product fractions.*—When representative inocula are desired from both the liquid and solid parts, transfer the solid component of the sample to the culture medium with forceps sterilized by flaming and use culture media in large test tubes (100×25 mm.) or in wide-mouthed jars or bottles.

C. CULTURE MEDIA

Because of their acid nature many products in this class are subjected to the limited processing designed only to prevent spoilage. This is done to preserve the color, flavor, and texture of the foods. In some instances the products are filled into the cans while hot and receive no further heat processing. Two main groups of microorganisms encountered in the spoilage of this class of canned food products are the aciduric bacteria and the yeasts. Viable bacteria capable of producing spoilage in non-acid foods but rendered inactive in acid foods have frequently been responsible for the reporting of false positive results in examinations for spoilage in acid canned foods. Use the following media adjusted to a reaction below *p*H 5.0 for the detection of spoilage organisms:

1. *Aciduric spoilage bacteria.*

Acid Meat Medium

Distilled water	1000 cc.
	grams
Ground fresh lean beef	500
Proteose peptone	5
C. P. NaCl	5
C. P. Dextrose	10

Infuse the beef-water mixture overnight in the refrigerator. Heat in Arnold or boil for 30 minutes. Strain through several layers of cheese cloth and press out broth, retaining the meat press cake. Add distilled water to the infusion to make up to 1 liter. Add the peptone and heat in Arnold or boil 10 minutes. Filter, and add salt. Acidify with C. P. lactic acid to *p*H 4.7, add the dextrose, and filter. Distribute the pressed-out beef remaining from the infusion into medium sized test tubes (150×20 mm.), approximately 2 grams into each tube, and add 10 cc. of the broth. Sterilize in the autoclave at 15 lbs. pressure for 15 minutes (final reaction should be *p*H 4.8). Prior to using, boil the tubed medium for 10 minutes to expel absorbed oxygen and cool promptly in a water bath.

As an alternative enrichment medium use digest-yeast-tomato juice, described in last year's report, modified by the addition of 0.5 per cent proteose peptone and adjusted to *p*H 4.8–5.0 with C. P. lactic acid.

The preparation of plate cultures is optional, and when desirable add 2% agar to the broth formula given above, dissolve by heating, filter, and adjust reaction to give final *p*H 5.0. Sterilize in an autoclave at 15 lbs. pressure for 15 minutes.

2. *Bacteria inhibited below pH* 5.0.—Use a medium essentially the same as the acid meat medium with reaction adjusted to *p*H 7.2. (This medium, when rid of excess free oxygen by boiling and prompt cooling just prior to use, has been found satisfactory for the growth of anaerobes, aerobes, and facultative bacteria.

3. *Yeast spoilage.*

Clarified malt extract medium, *This Journal,* 19, 445 (1936).

Dry malt extract (Difco)	100 grams
Distilled water	1000 cc.

Dissolve the powdered malt extract in the water by heating in an Arnold sterilizer, or on the water bath. Adjust to *p*H 4.7 and cool to 50° C. Add slowly 100 cc. of a 5% suspension of Bentonite (colloidal clay) and mix vigorously. Hold at 50°–75° C. for 30 minutes, then filter through a fluted paper filter until clear. Heat the filtrate in the autoclave 10 minutes at 15 lbs. pressure, and filter through paper to remove any precipitate formed. Distribute into tubes, or flasks. For a plating medium, dissolve by heating, 2% agar-agar in the clarified broth, and filter if necessary through cotton and cheese cloth. To avoid further precipitation sterilize at 10 lbs. pressure for 15 minutes and cool promptly. (Bacto Malt Extract Broth can now be obtained in convenient dehydrated form and may be substituted for the above medium.)

4. *"Flat-sour" spoilage bacteria.*

Medium for detection of "flat-sour" organisms

The bromocresol purple dextrose tryptone medium, recommended by Cameron in his Report on Culture Media for Non-Acid Products, *This Journal,* 19, 433 (1936), has been found useful in the detection of thermophilic bacteria occurring in acid canned foods.

D. INCUBATION

Incubate all cultures for the detection of spoilage organisms for at least 72 hours at 30° C. For the detection of non-aciduric bacteria, incubate at 37.5° C. for at least 48 hours. For the occasional "flat-sour" spoilage encountered in such products as tomato juice, incubate for thermophilic anaerobes at 56° C. for at least 48 hours.

E. CULTURE STUDY

Use the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists as a guide for study of microorganisms obtained in the cultural procedure described.

REPORT ON METHODS FOR DETECTING AND ESTIMATING NUMBERS OF THERMOPHILIC BACTERIA IN SUGAR

By E. J. Cameron (Research Laboratories, National Canners Association, Washington, D. C.), *Associate Referee*

The report on sugar methods, made in 1935, and published in *This Journal,* 19, 439 (1936), included methods for the three types of thermophilic spoilage bacteria that may cause spoilage in canned foods. Included was the group of sulfide spoilage organisms, otherwise described as thermophilic anaerobes that produce hydrogen sulfide. Sulfite agar, the test medium for this group, was described in a previously published Report on Culture Media for Non-Acid Products, *Ibid.,* 434.

Yeast water is the base for sulfite agar. It is prepared by autoclaving at 15 pounds' pressure for 5 hours a 10 per cent suspension of starch-free yeast and allowing several days for settling. Complaints have been made that the preparation of yeast water is too time-consuming for a laboratory in which tests for sulfide organisms are made infrequently. Yeast extract has been substituted for yeast water with but indifferent results.

The following medium has been used with complete success in this laboratory and has the advantage of being easily prepared. Comparative tests on this and the original medium are much in favor of the new medium, which is described as follows: Water—1 liter, tryptone—10 grams, Na_2SO_3—1 gram, agar—20 grams. At the time of tubing, a clean iron strip or nail is placed in the tube. No adjustment in reaction is necessary. As in sulfite agar, the "sulfide" spoilage organisms are detected through the formation of characteristic blackened spherical areas. In the usual case there is no gas formation. The presence of gas coupled with a general blackening of the medium indicates the presence of thermophilic anaerobes not of the sulfide spoilage group. The darkening of the medium in this case results from the reduction by hydrogen gas.

This medium is now recommended as the principal medium for the detection of sulfide spoilage organisms.

ASSOCIATE REFEREE APPOINTED

K. L. Milstead, U. S. Food and Drug Administration, Chicago, Ill., has been appointed Associate Referee on Standardization of Iodine and Thiosulfate Solutions.

CORRECTION

Methods of Analysis, A.O.A.C., 1935.—On p. 322, section **15**, 3rd line from end of paragraph, change "100 cc." to "10 cc."

CONTRIBUTED PAPERS

TITRATION OF SMALL QUANTITIES OF FLUORIDES WITH THORIUM NITRATE

I. EFFECT OF CHANGES IN THE AMOUNT OF INDICATOR AND ACIDITY

By DAN DAHLE, R. U. BONNAR, and H. J. WICHMANN (Food Division,* U. S. Food and Drug Administration, Washington, D. C.)

The use of aqueous instead of alcoholic solutions for the titration of fluorine with thorium nitrate, as proposed by W. D. Armstrong,[1] was applied to comparatively large amounts of fluorine (0.5–10 mg.) by Rowley and Churchill.[2] These authors state that in this range a *p*H of 2.9–3.1 is an optimum, that for small amounts of fluorine the titration is very sensitive to *p*H, and that the optimum is unknown.

The writers consider that much of the trouble encountered in titrating small quantities of fluorine can be eliminated by the adoption of a suitable modification of the following "back titration procedure," suggested by W. S. Allen:[3]

METHOD

REAGENTS

Thorium solution.—Dissolve 0.25 gram of $Th(NO_3)_4 \cdot 12H_2O$ in 1 liter of water (exact strength not important).

Sodium fluoride.—1 cc. = 0.01 mg. of fluorine. Prepare by diluting a stock solution containing 1 gram of fluorine (2.22 grams of NaF of 98–99% purity) per liter.

Indicator.—A 0.01 per cent water solution of alizarin red S (sodium alizarin sulfonate).

Hydrochloric acid.—0.05 *N* (approximately 1 cc. of concentrated HCl in 250 cc.).

Sodium hydroxide.—0.05 *N* (2 grams of NaOH per liter).

APPARATUS

Nessler tubes.—50 or 100 cc. volume (Kimball Shadowless 50 cc. tubes were found to be satisfactory).

PROCEDURE (USING 50 CC. TUBES)

Place 40 cc. of sample solution in a 50 cc. Nessler tube. Also prepare a blank tube with distilled water. Add to each tube 1 cc. of alizarin red indicator, and mix. To the sample tube add the NaOH dropwise, with mixing, until its color just matches that of the blank, which is faintly pink (neutral). To each tube add *exactly* 2.00 cc. of the HCl. Mix well.

From a 10 cc. buret, graduated in 0.05 cc., add the thorium solution to the sample tube until, after mixing, the color barely changes to faint pink. Note carefully the amount added and add *exactly* the same amount to the blank. Mix.

Into the blank (now more highly colored than the sample tube), run the NaF from another 10 cc. buret until the colors of the two tubes match (after dilution to the same volume). Mix well, and allow all air bubbles to escape before making the

* W. B. White, Chief.
[1] *Ind. Eng Chem Anal. Ed.*, **8**, 384 (1936).
[2] *Ibid.*, **9**, 551, (1937).
[3] Private communication, published by permission.

final color comparison. Check the end point by adding 1–2 drops of the NaF to the blank. (A distinct color change should develop.)

The fluorine content of the sample tube equals the amount added to the blank tube.

The following study was made of causes for "off" results in titration of small amounts of fluorine. It was not concerned with quantities larger than 125 micrograms of fluorine per 100 cc., and results within ± 0.5 microgram of each other were considered identical.

1. *Effect of the Manner of Adding Thorium Nitrate*

Since the color at the end point of the titration is due to the formation of a thorium-alizarin lake, it was considered to be conceivable that adding a certain volume of thorium solution dropwise with mixing after each drop, and adding the same quantity all at one time, might result in a different color, due to a difference in the dispersion of the lake formed. Repeated experiments indicated a tendency toward more color when all the thorium solution was added at one time. In no case, however, did the difference correspond to more than 0.5 microgram of fluorine, as checked by the addition of this amount of fluorine to the more highly colored tube.

Incidentally the same bleaching effect on the more highly colored tubes could be obtained by breathing carbon dioxide into them for 10–20 seconds.

It seems justifiable to conclude, therefore, that the thorium-alizarin lake disperses in practically the same manner, whether the thorium solution is added dropwise or in one portion. (This conclusion, of course, cannot be depended upon to hold for all concentrations.)

2. *Effect of Varying Amounts of Indicator*

It has long been recognized that the amount of indicator used influences the result. Instead of 3 drops of the 0.05 per cent solution, originally suggested by Armstrong,[1] the present studies were made with measured amounts of a 0.01 per cent indicator solution.

In these experiments paired tubes were used in determining (1) the amount of thorium solution needed to produce the pink color, and (2) the amount of fluorine as sodium fluoride that would cause a visible change in the color so produced. Table 1 gives the results.

It appears that the blank, *i.e.* the amount of thorium solution needed to produce a pink color in the absence of fluorine, increases as the amounts of indicator used are increased (Table 1). In tubes 7–10 a tan color (mixture of pink and yellow) made it rather difficult to decide on the end point, both in the direct titration with thorium solution and in the back titration with sodium fluoride. Tubes 1–2, on the other hand, might have been

[1] *J. Am. Chem. Soc.*, 55, 1741 (1933).

TABLE 1.—*Effect of variations in the amount of indicator*

TUBE NO.	INDICATOR USED	THORIUM SOLUTION NEEDED FOR—		NaF ADDED		REMARKS
		INCIPIENT PINK	DECIDED PINK	1ST CHANGE	INDISPUTABLE CHANGE	
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	
1– 2	0.5	0.30	0.35	0.03	0.05	Rather pale
3– 4	1.0	0.35	0.45	0.03	0.05	O.K.
5– 6	2.0	0.45	0.60	0.05	0.07	O.K.
7– 8	3.0	0.55	0.75	0.05	0.10	Poor end point
9–10	4.0	0.65	0.95	0.10	0.15	Poor end point

considered by some analysts too pale for a good color match. The optimum appears to be 1 cc. of a 0.01 per cent indicator solution per 50 cc. volume, with 0.5 and 2 cc. as optional.

These experiments were repeated with 20 micrograms of fluorine present. The blank tubes were titrated to incipient pink color and the "fluorine tubes" titrated to the same color. The results appear in Table 2.

TABLE 2.—*Effect of indicator in the presence of fluorine*

TUBE	INDICATOR USED	THORIUM SOLN ADDED	THORIUM SOLN REQUIRED BY 20 MICROG. OF FLUORINE	FLUORINE PER 1 CC. OF THORIUM SOLN
	cc.	*cc.*	*cc.*	*micrograms*
A_b	0.5	0.30	1.60	12.50
A_f	0.5	1.90		
B_b	1.0	0.40	1.40	14.29
B_f	1.0	1.80		
C_b	2.0	0.60	1.15	17.39
C_f	2.0	1.75		
D_b	3.0	0.75	1.00	20.00
D_f	3.0	1.75		
E_b	4.0	0.85	0.95	21.05
E_f	4.0	1.80		

Table 2 shows that the titer of the thorium nitrate increases with increasing amounts of indicator. A graphical expression of this relation-

ship (omitted for the sake of brevity) would show that the titer varies directly with the logarithm of the indicator concentration. This variation in the titer emphasizes the need for precise measurements of the indicator.

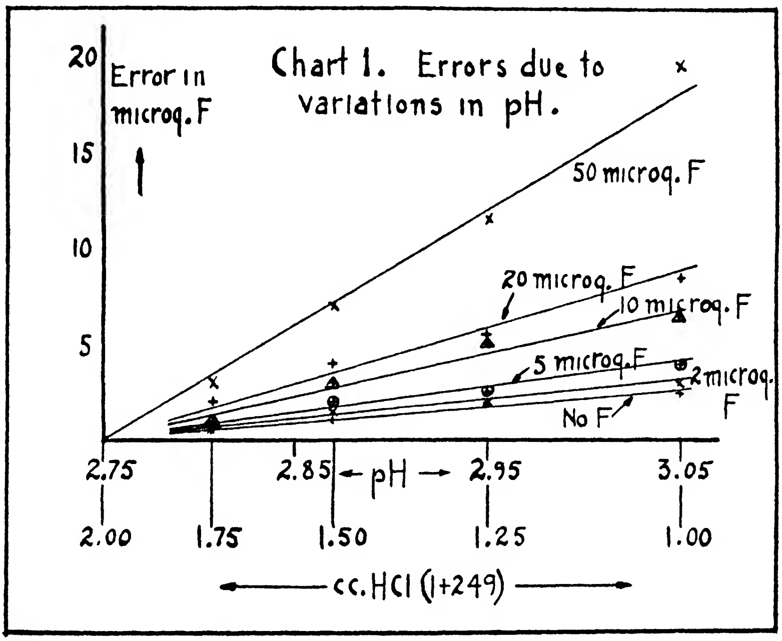
Incidentally, when the *same amounts* of thorium solution were added to blanks and fluorine tubes containing identical amounts of indicator and subsequent back titrations were made with sodium fluoride, the results were all within ±0.5 microgram of the 20 micrograms of fluorine added to the fluorine tubes.

TABLE 3.—*Effect of variations in pH*

SERIES	FLUORINE ADDED	HCl (1+249) ADDED	THORIUM SOLUTION ADDED	NaF USED
	micrograms	*cc.*	*cc.*	*cc.*
	none	2.00	0.45	0
	none	1.75	0.45	0.05
A	none	1.50	0.45	0.12
	none	1.25	0.45	0.20
	none	1.00	0.45	0.25
	2	2.00	0.55	0
	2	1.75	0.55	0.05
B	2	1.50	0.55	0.15
	2	1.25	0.55	0.20
	2	1.00	0.55	0.30
	5	2.00	0.70	0
	5	1.75	0.70	0.10
C	5	1.50	0.70	0.20
	5	1.25	0.70	0.25
	5	1.00	0.70	0.40
	10	2.00	1.10	0
	10	1.75	1.10	0.10
D	10	1.50	1.10	0 30
	10	1.25	1.10	0.50
	10	1.00	1.10	0.65
	20	2.00	1.60	0
	20	1.75	1.60	0.20
E	20	1.50	1.60	0.40
	20	1.25	1.60	0.55
	20	1.00	1.60	0.85
	50	2.00	3.20	0
	50	1.75	3.20	0.30
F	50	1.50	3.20	0.70
	50	1.25	3.20	1.15
	50	1.00	3.20	1.95

3. *Effect of Variations in the Acidity*

To 40 cc. aliquots of solutions, containing the same amount of indicator and sodium fluoride, were added 1.00, 1.25, 1.50, 1.75, and 2.00 cc. of approximately 0.05 N hydrochloric acid (1 cc. conc. HCl+249 cc. water). Next, thorium solution was added to the 2 cc. tube to incipient pink color. The *same amount* of thorium solution was then added to each of the other tubes in the series. The resultant colors ranged from faint pink in the 2 cc. tube to reddish in the 1 cc. tube. Back titrations were then made with sodium fluoride, until all tubes matched the 2 cc. tube. The results of these back titrations give a measure of the error introduced by variations



in the pH from 2.75 (the 2 cc. tube) to 3.05 (the 1 cc. tube). These experiments were performed with distilled water and also with distillates taken over at 130–135° C. from a mixture of 20 cc. of 60 per cent perchloric acid and 10 cc. of water. Table 3 gives the results for distillates. The water solutions gave similar data.

The data in Table 3 are expressed graphically in Chart 1. From this chart it appears—

(1) That a change in pH from 2.75 to 3.05, in the absence of fluorine, would cause an error of 2.5 micrograms. As the fluorine content of the solution increases, however, the error, caused by variations in pH, also increases.

(2) That the error, within the limits studied, is directly proportional to the change in *p*H.

The experiment was repeated with 1, 2, 3, 5, and 10 cc. of hydrochloric acid in the tubes, and the thorium solution added to each tube was the amount needed to give a pink color in the 10 cc. tube. In this case, too, the error was found to be (approximately at least) proportional to the change in *p*H.

These data indicate that a lower *p*H is preferable, since the change in *p*H caused by a given amount of acid at *p*H 2 is only one-tenth of the change caused by the same amount of acid at *p*H 3.

The next experiment was carried out as follows: Paired tubes were prepared (zero and F), containing the same amount of indicator and hydrochloric acid, but fluorine was added in one tube (F) only. First the zero-tubes received thorium solution to incipient pink and then the F-tubes were titrated to the same color. This gave the titer of the thorium solution. Next the zero-tubes received additional thorium solution to equal concentration of that in the F-tubes, and a back titration with sodium fluoride was made. This gave a measure of the precision of the back titration at different levels of acidity. The results are found in Table 4.

TABLE 4.—*Effect of acidity on titer of thorium solution*

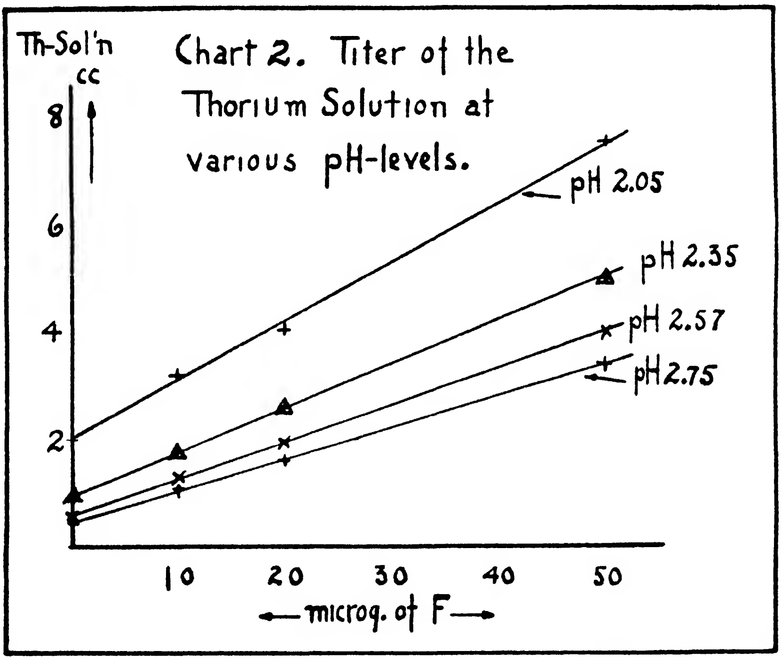
SERIES	FLUORINE ADDED	THORIUM SOLN ADDED	TITER OF THORIUM SOLN	NaF USED	FLUORINE FOUND	ERROR
	micrograms	*cc.*	*microg. F./cc.*	*cc.*	*micrograms*	*microgram*
A	0	0.40	—	0.05	0.5	+0.5
2 cc. HCl	10	1.05	15.4*	0.95	9.5	−0.5
*p*H 2.75	20	1.60	16.7	—	—	—
	50	3.40	16.7	4.95	49.5	−0.5
B	0	0.55	—	0.05	0.5	+0.5
3 cc. HCl	10	1.30	13.3	1.00	10.0	±0
*p*H 2.57	20	1.95	14.3	—	—	—
	50	4.00	14.5	4.95	49.5	−0.5
C	0	0.95	—	−0.05	0.5	−0.5
5 cc. HCl	10	1.75	12.5	+0.95	9.5	−0.5
*p*H 2.35	20	2.60	12.1	—	—	—
	50	5.00	12.3	5.00	50.0	±0
D	0	2.00	—	0.10	1.0	+1.0
10 cc. HCl	10	3.20	8.3	1.05	10.5	+0.5
*p*H 2.05	20	4.05	9.8	—	—	—
	50	7.55	9.0	5.10	51.0	+1.0

* The titer of the thorium solution was calculated as follows: 0.40 cc. thorium solution needed to color zero-tube, 1 05 cc. needed to give the same color to F-tube. Difference (1.05–0.40) = 0.65 cc. taken up by 10 micrograms of fluorine. Titer: 10/0.65 = 15.4 micrograms. F/cc. thorium solution.

Chart 2 shows these results graphically. From Chart 2 and Table 4 it appears—

(1) That the titer of the thorium solution varies directly with the *p*H at which it is determined.

(2) That for the same *p*H the titer is independent of the amount of fluorine used for the titer determination. This corroborates the findings of Armstrong,[1] but (as he also stated) it does not hold true when titrations are made in the alcoholic mixture originally suggested by Willard and Winter.[2]



(3) That back titrations with sodium fluoride can be made with equal precision in the presence of 2, 3, and 5 cc. of 0.05 hydrochloric acid. The precision, however, decreases with greater acidities. In fact, even with 5 cc. of hydrochloric acid the changes per drop of sodium fluoride are less apparent than at lower acidities.

It would seem, therefore, that a *p*H below 2.5 is less suitable because of decreased precision in the titration.

Of interest is a comparison of the change in titer (Δ Titer) with change in *p*H (Δ *p*H), which is shown in Table 5.

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[1] *Ind. Eng. Chem. Anal. Ed.*, **8**, 384 (1936).
[2] *Ibid.*, **5**, 7 (1933).

TABLE 5.—*Variation of titer with pH*

HCl ADDED	AV. TITER (TABLE 4)	Δ TITER	pH	Δ pH	Δ TITER PER 0.1 Δ pH
cc.	*microg. F./ cc. Th*	*microg. F./ cc. Th*			*microg. F./cc. Th/ 0.1 pH unit*
2	16.27		2.75		
3	14.03	2.24	2.57	0.18	1.3
5	12.30	3.97	2.35	0.40	1.0
10	9.04	7.23	2.05	0.70	1.0
				Average	1.1

Thus in two solutions of the same fluorine content, but varying in *p*H by 0.02 units, titrations for fluorine may be expected to differ by $(0.02/0.1)\times 1.1$, or 0.22 microgram *for each cc. of thorium solution used in the titration.*

This deduction, then, supplies another limiting factor for the optimum *p*H. If a low *p*H, *e.g.* 2.57, is chosen, a total of 4 cc. of thorium solution is used for a titration of 50 micrograms of fluorine (cf. Table 4). The error caused by a difference of 0.02 *p*H units would become 0.88 microgram, or 1.76 per cent. At *p*H 2.75 the corresponding error would become 1.50 per cent and at *p*H 3.05, 1.23 per cent. On the other hand, a difference of 0.02 in the *p*H would be caused by approximately 0.05 cc. of 0.05 *N* hydrochloric acid at *p*H 3.05 and by 0.12 cc. of 0.05 *N* hydrochloric acid at *p*H 2.57.

The next consideration is the reaction during the titration. If it is assumed that the postulate of Willard and Winter (*loc. cit.*), that H_2SiF_6 is the fluorine combination present in the distillate, is correct then

$$2\,H_2SiF_6 + 3\,Th(NO_3)_4 = 3\,Th\,F_4 + 12\,HNO_3 + 2\,SiO_2.$$

Thus, in the presence of 50 micrograms of fluorine as H_2SiF_6, 0.0632 mg. or 0.017 cc. of 0.05 *N* H_2SiF_6 disappears, while 0.166 mg. or 0.05 cc. of 0.05 *N* HNO_3 is formed during the titration. The difference is 0.033 cc. of 0.05 *N* acid.

It is evident, therefore, that the next limiting factor is the error caused by the acid formed during the titration. Hence a *p*H must be chosen at which 0.033 cc. of 0.05 *N* acid fails to cause an appreciable change in the *p*H. (A difference between nitric and fluosilicic acid in regard to hydrogen ion activity may surely be disregarded at these dilutions.)

The upper limit, therefore, seems to be *p*H 3 and the lower limit, *p*H 2.5. Probably *p*H 2.75 would be as good a practical choice as any.

4. *Effect of Variations in the Volume of Liquid*

Since variations of the indicator and of the acid concentrations affect the titer of the thorium solution, it is apparent that best results may be obtained only when the volume of liquid in the tube containing the unknown equals that in the tube used for comparison. Since, however, an increase in the acid concentration affects the thorium-fluorine reaction in the same manner as does a decrease in the indicator concentration, a small change of both in the *same* direction should cause no great error. Experiments made at different acidities gave the results shown in Table 6.

TABLE 6.—*Effect of volume changes*

*p*H	FLUORINE PRESENT	VOLUME CHANGE	ERROR
	micrograms	*cc.*	*micrograms*
3.05	0	5	0–0.3
3.05	50	5	0.3–0.7
2.75	0	5	0–0.3
2.75	50	5	0.3–0.5
2.57	0	5	0.3–0.5
2.57	50	5	0.5–1.0
2.35	0	5	0.3–0.5
2.35	50	5	1.0–2.0
2.05	0	5	0.5–1.0
2.05	50	5	2.0–3.0

Evidently the "volume error" is increased as the *p*H is lowered. Within the range 2.5–3.0, however, a volume difference of 10 per cent (5 cc. in 50) seems to cause no appreciable error.

SUMMARY

A titration procedure for the determination of small quantities of fluorine is described. Thorium nitrate is the reagent used.

The comparison made between the additions of thorium solutions dropwise and all at one time shows that the resultant color of the lake is practically the same in each case.

Variations in the amount of indicator used resulted in different values for the titer of the thorium solution and in a difference in the size of the blank.

Variations in the acidity of the solution to be titrated for fluorine caused (1) varying size of the blank, (2) varying titer of the thorium solution, and (3) errors approximately proportional to the change in *p*H and the amount of fluorine present.

A suitable *p*H range (2.5–3.0) is suggested for titrations of quantities up to 50 micrograms of fluorine.

TITRATION OF SMALL QUANTITIES OF FLUORIDES WITH THORIUM NITRATE

II. EFFECTS OF CHLORIDES AND PERCHLORATES

By Dan Dahle, H. J. Wichmann, and R. U. Bonnar (Food Division,* U. S. Food and Drug Administration, Washington, D. C.)

The effect of variation in acidity on the result of the titration for fluorine has been discussed by the same writers in a previous paper (see p. 459). Usually the titration is preceded by an isolation by distillation from perchloric, sulfuric, or phosphoric acid. D. S. Reynolds,[1] and later Churchill, Bridges, and Rowley[2] have called attention to the tendency of phosphoric acid to distil over, thus causing plus errors in the fluorine titration, since it reacts with thorium similarly to fluorine. W. D. Armstrong[3] has showed that perchloric acid is partly volatile under the conditions of the distillation and suggested the addition of sodium perchlorate to the liquid in the distilling flask in order to keep the acidity of the distillate low.

Since perchloric acid is present in the distillate, its influence on the result of the fluorine titration is of interest. Hoskins and Ferris[4] state that a 0.1 *M* concentration of perchloric acid is needed to produce an appreciable effect. This would mean about 500 mg. (100 cc.) of 0.05 *N* acid in 50 cc. of distillate. On the other hand, the efforts of Armstrong (*loc. cit.*) to lower the perchloric acid content of his distillates indicate interference at lower concentrations. The work of Reynolds and Hill[5] throws light on this point. They show that the errors caused by interferences vary with the amount of fluorine present. In a recent paper Eberz, Lamb, and Lachele[6] indicate (Table 1) that the error caused by sulfuric acid increases as the amount of fluorine in the distillate increases.

These observations indicate that some negative ions other than fluorine change the titer of the thorium solution either through a *partial* formation of an undissociated salt of thorium and the negative ion in question, or in some other manner.

1. *Perchloric vs. Hydrochloric Acid*

(For reagents and method used in this and subsequent experiments, see preceding paper by the same authors.)

This experiment aimed at testing possible differences in the titration results when perchloric was substituted for hydrochloric acid. Sets of

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* W. B. White, Chief.
[1] *This Journal*, 18, 110 (1935).
[2] *Ind. Eng. Chem. Anal. Ed.*, 9, 222 (1937).
[3] *Ibid.*, 8, 384 (1936).
[4] *Ibid.*, 6.
[5] In publication.
[6] *Ind. Eng. Chem. Anal. Ed.*, 10, 259 (1938).

paired Nessler tubes were used, each member of the pair containing the same amount of indicator, fluorine, and thorium solution. One of the tubes in the pair was made acid with 2 cc. of 0.05 *N* hydrochloric acid and the other received 2 cc. of 0.05 *N* perchloric acid. If color differences were apparent, the darker tube received standard sodium fluoride solution until both tubes appeared to be equal. The amount thus added gave a measure of the error (plus or minus) due to the use of perchloric instead of hydrochloric acid.

To check the end point in the color comparison additional sodium fluoride was added until an indisputable difference was noticeable between the two tubes.

Table 1 shows the results.

TABLE 1.—*Effect of perchloric acid*

TUBE NO.	0.05 *N* ACID	FLUORINE ADDED	THORIUM SOLUTION ADDED	NaF USED FOR— COLOR MATCH	INDISPUTABLE CHANGE	ERROR CAUSED BY USE OF $HClO_4$
	cc.	*micrograms*	*cc.*	*cc.*	*cc.*	*microgram of F*
1	1-HCl	0	0.25	—	—	
2	1-$HClO_4$	0	0.25	—	0.03	none
3	1-HCl	50	2.55	—	—	
4	1-$HClO_4$	50	2.55	—	0.03	none
5	2-HCl	0	0.30	—	—	
6	2-$HClO_4$	0	0 30	—	0.03	none
7	2-HCl	50	3.20	—	—	
8	2-$HClO_4$	50	3.20	—	0.03	none
9	3-HCl	0	0.35	—	—	
10	3-$HClO_4$	0	0.35	0.03	0.05	−0.3
11	3-HCl	50	3.80	—	—	
12	3-$HClO_4$	50	3.80	0.05	0 07	−0 5
13	5-HCl	0	0.50	—	—	
14	5-$HClO_4$	0	0.50	0.05	0.07	−0.5
15	5-HCl	50	4.75	—	—	
16	5-$HClO_4$	50	4.75	0.07	0.12	−0.7
17	10-HCl	0	2.00	—	—	
18	10-$HClO_4$	0	2.00	0 05	0.15	−0.5
19	10-HCl	50	6.85	—	—	
20	10-$HClO_4$	50	6.85	0.05	0.15	−0 5

Table 1 indicates that within the limits of the experiment no appreciable error is caused in the titration results by the substitution of perchloric for hydrochloric acid. The apparent errors found at acid concentrations of 5 and 10 cc. may be within the limits of experimental error. In any event, it will be noted that in each case they are *minus* errors.

Repetition of these experiments with partial instead of complete substitution of perchloric acid led to the same conclusions.

2. *Effect of Chlorides and Perchlorates*

Two neutral 0.05 *N* solutions, one of sodium chloride and one of sodium perchlorate, were prepared, and the effect of the presence of varying quantities of these salts was studied. Table 2 gives the results.

TABLE 2.—*Effect of NaCl and $NaClO_4$*

SERIES	0.05 *N* HCl	SALT SOLUTION	FLUORINE ADDED	THORIUM SOLUTION	NaF	ERROR CAUSED BY SALT
	cc.	*cc.*	*micrograms*	*cc.*	*cc.*	*microgram of F*
A	2	0	0	.30	—	none
	2	1-NaCl	0	.30	—	
B	2	0	0	.30	—	none
	2	1-$NaClO_4$	0	.30	—	
C	2	0	50	3.35	0.05	+0.5
	2	1-NaCl	50	3.35	—	
D	2	0	50	3.35	0.15	+1.5
	2	1-$NaClO_4$	50	3.35	—	
E	2	0	0	0.35	—	none
	2	5-NaCl	0	0.35	—	
F	2	0	0	0.35	0.05	+0.5
	2	5-$NaClO_4$	0	0.35	—	
G	2	0	50	3.45	0.25	+2 5
	2	5-NaCl	50	3.45	—	
H	2	0	50	3.50	0.30	+3.0
	2	5-$NaClO_4$	50	3.50	—	
I	2	0	0	0.40	0.05	+0.5
	2	10-NaCl	0	0.40	—	
K	2	0	0	0.40	0.10	+1.0
	2	10-$NaClO_4$	0	0.40	—	
L	2	0	50	3.70	0.35	+3.5
	2	10-NaCl	50	3.70	—	
M	2	0	50	3.65	0.45	+4.5
	2	10-$NaClO_4$	50	3.65	—	

These experiments were repeated, hydrochloric acid and perchloric acid, respectively, being added and neutralized directly in the Nessler tube. The results were the same as those given in Table 2.

It thus appears that the presence of chlorides and/or perchlorates affects the titration and is a cause of plus errors in the fluorine determination. The error increases as the amount of fluorine in the solution in-

creases, but the results in Table 1 show that it is not due to the perchlorate (or chloride) ion. The error may, therefore, be due either to some change in the reaction between thorium, fluorine, and the indicator, or, more probably, to the effect of salts on the *p*H, alizarin sulfonate being an indicator rather sensitive to salt effects.[1]

This brings up the question: "How would the use of the Hoskins-Ferris buffer affect this salt error?" Table 3 shows the results of experiments when paired tubes were used, one with and the other without sodium perchlorate.

TABLE 3.—*Salt error in the presence of buffer*

EXPERIMENT NO.	FLUORINE PRESENT	BUFFER USED	0.05 *N* $NaClO_4$	THORIUM SOLUTION	NaF	ERROR DUE TO $NaClO_4$
	micrograms	*cc.*	*cc.*	*cc.*	*cc.*	*microgram*
A	0	1	0	0.75	0.03	
	0	1	5	0.75		+0.3
B	50	1	0	3.25	0.20	
	50	1	5	3.25	—	+2.0
C	0	1	0	0.65	0.15	
	0	1	10	0.65	—	+1.5
D	50	1	0	3.80	0.40	
	50	1	10	3.80	—	+4.0
E	20	0 25	0	1.00	0.05	
	20	0.25	5	1.00	—	+0.5
F	20	0.50	0	1.35	0 07	
	20	0.50	5	1.35	—	+0.7
G	20	2.0	0	2.25	0.10	
	20	2.0	5	2.25	—	+1.0
H	20	5.0	0	5.00	?-0.50	up to 5.0
	20	5.0	5	5.00		

It appears that the use of Hoskins-Ferris buffer does not eliminate the "salt error" (Table 3). It was noticed, however, that the color of the indicator at the end point was paler and that the change per drop of fluorine solution was decidedly less noticeable in the presence of buffer. This effect was similar to the one produced by the presence of large amounts of salts.

Since Tables 1 and 2 show that the presence of perchloric acid will cause errors *only if the acid is neutralized,* the following procedure was tried out:

[1] Clark, The Determination of Hydrogen Ions, 3rd ed., p. 183. Williams & Wilkins Co., Baltimore (1928).

The acidity of 40 cc. of distillate was determined by titration with 0.05 *N* sodium hydroxide, and alizarin red was used as an indicator. To another 40 cc. aliquot of distillate was added enough 0.05 *N* hydrochloric acid to make the *total* acidity equal 2 cc. of 0.05 *N* acid per 40 cc. The fluorine content of the aliquot was then determined by the "back titration procedure," previously described by Dahle, Bonnar, and Wichmann (see p. 459). Table 4 shows the results.

TABLE 4.—*Titrations without neutralization*

SAMPLE NO.	ACIDITY OF 40 CC. 0.05 *N*	0.05 *N* HCl ADDED	FLUORINE ADDED AFTER DISTILLATION	THORIUM-NITRATE USED	NaF USED	FLUORINE ADDED	FLUORINE FOUND	ERROR
	cc.	*cc.*	*micrograms*	*cc.*	*cc.*	*micrograms*	*micrograms*	*micrograms*
1	0.40	1.60	0	0.40	0.05	0	0.5	+0.5
2	0.40	1.60	50	3.35	5.05	50	50.5	+0.5
3	1.25	0.75	0	0.40	0.07	0	0.7	+0.7
4	1.25	0.75	50	3.35	5.07	50	50.7	+0.7
5	4.35	—	0	0.75	0.12	0	1.2	+1.2
6	5.25	—	0	0.85	0.07	0	0.7	+0.7
7	8.50	—	0	2.10	0.07	0	0.7	+0.7
8	8.50	—	50	5.50	5.10	50	51.0	+1.0

Compared with the results in Table 1, the results in Table 4 seem to indicate an apparent fluorine content of the distillate. This is in line with previous findings by Dahle[1] and will be treated in greater detail later. Apparently, however, the titrations without previous neutralization give correct results on added known amounts of fluorine even at rather high acidities.

Finally a comparison was made between aliquots of the same distillate treated by the usual neutralization procedure and by the "acid substitution" method used in the previous experiment. The results in each case showed that for the same color the "neutralized tube" required more thorium solution than the non-neutralized one. The difference, as determined by addition of sodium fluoride to the darker tube, increased with increasing acidity of the distillate, *i.e.* with the amount of salts present.

It appears, therefore, that the neutralization of the acid in the distillate before titration of the fluorine with thorium solution introduces a plus error of variable size. This error apparently is caused by alkali salts of the acid present in the distillate. Correct results on added known quantities of fluorine can be obtained if neutralization is avoided, and the sample and standard are compared at equal acidities. This, however, does not hold true when the acid in the distillate is sulfuric or phosphoric, since these acids cause added errors due to formation of undissociated salts with thorium.

[1] *This Journal*, 21, 208 (1938).

3. *Elimination of Acidity in the Distillate*

The preceding experiments indicate that the acidity problem comes down to finding a means of eliminating *excessive* quantities of perchloric acid in the distillate. It has been previously shown (see p. 437) that 250 cc. of distillate from perchloric acid has been known to require from 0.6 to 300 cc. of 0.05 *N* alkali for neutralization. (For sulfuric acid, 200 cc. of distillate required 0.4 to 4.5 cc. of 0.05 *N* alkali.)

Experiments have also shown that with the same perchloric acid-water mixture the volatilization of the acid increases with the size of the flask and with increased temperature of distillation. Table 5 shows a comparison.

TABLE 5.—*Temperature vs. acidity*

TEMPERATURE	0.05 *N* ALKALI PER 50 CC. DISTILLATE	REMARKS
°*C.*	*cc.*	
110	0.10	These distillations were made in
120	1.30	an all-glass apparatus; the
130	3 15	size of the distilling flask was
140	4.45	250 cc.

Further experiments showed that this volatility of a "non-volatile" acid is due to the overheating of the walls of the distilling flask above the surface of the liquid (verified by touching a thermometer to the outside of the flask during the distillation).

The volatilization of perchloric acid can be greatly decreased by exposing less surface of the flask to the flame and by shielding the sides of the flask from contact with the flame or overheated air. This may be done by resting the flask on an 8″×8″ pad of transite or asbestos, with a hole about 2 inches in diameter, *made to fit the flask perfectly.* Table 6 gives comparative data showing the influence of such shielding. In this experiment a mixture of 20 cc. of 60 per cent perchloric acid and 10 cc. of water was distilled in various sized flasks. Transite pads 4″×4″ and 8″×8″ were used. The original acid-water mixture was transferred from flask to flask, and the distillations were made in the order listed in Table 6.

TABLE 6.—*Effect of shielding on acidity*

TEMPERATURE	SIZE OF FLASK	0.05 *N* ACID/200 CC. DISTILLATE		REMARKS
		4″×4″	8″×8″	
°*C.*	*cc.*	*cc.*	*cc.*	
130	125	1.20	0.50	c.f. last line
130	250	10.10	0.45	
130	1000	57.00	4.35	
140	125	4.50	3.20	
140	250	52.00	6.00	
140	1000	118.60	44.20	
130	125	0.80	0.40	c.f. first line

The results in Table 6 point definitely to a way of eliminating excess acidity. A low acidity in the distillate would greatly decrease the neutralization error (c.f. Table 2). It also seems probable that a combination of low acidity distillates with the "acid substitution" procedure (c.f. Table 4) would lend itself to the development of a suitable titration method for very small quantities of fluorine. Preliminary experiments by the writers along this line indicate that when the amount of fluorine present is under 5 micrograms distinct indicator changes can be produced for less than 0.2 microgram of fluorine.

SUMMARY

The effect of chlorides and perchlorates on the titration of small quantities of fluorine with thorium nitrate was investigated. It appears—

(1) That the presence of these salts introduces a plus error, the size of which varies with the amount of salts present and with the amount of fluorine to be determined.

(2) That the use of the Hoskins-Ferris buffer does not eliminate this salt error.

(3) That correct results can be obtained if the neutralization of the distillate is avoided. A procedure is suggested for titration without neutralization.

The cause of high acidities in the distillates from the Willard-Winter distillation with perchloric acid was also investigated. A procedure is suggested for eliminating such high acidity by distilling below 140° C. and by shielding the flask to avoid overheating.

A possible development of a micro-titration is indicated.

DETECTION OF FENUGREEK EXTRACT IN ARTIFICIAL MAPLE FLAVOR

BY THE DETERMINATION AND OPTICAL IDENTIFICATION OF CHOLINE

By JOHN B. WILSON and GEORGE L. KEENAN*

For many years fenugreek seed (*Trigonella foenum-graecum*) has been used as a source of artificial maple flavor. The seeds themselves are not used as human food, but their alcoholic extract, either alone or in combination with vanilla, vanillin, coumarin, coffee extract, extract of lovage root, and other ingredients, is used to give an artificial maple flavor to cakes, pies, confectionery, and other food products. The ground seeds are also frequently found in cattle feeds.

A search of the literature does not reveal the identity of the flavoring substances of fenugreek seed that give the maple-like character, although

* Joint contribution from the Beverage Section, Food Division, and the Microanalytical Division, Food and Drug Administration, U. S. Department of Agriculture.

it is stated by Hare, Caspari, and Rusby[1] that "the odorous principle is derived from mannogalactane." E. Jahns[2] reports the presence of 0.05 per cent choline, $(CH_3)_3 \cdot N(OH) \cdot CH_2CH_2OH$, in the seeds.

Although it has been known for many years that choline is a constituent of fenugreek seed, J. L. Perlman of the Department of Agriculture and Markets of the State of New York was the first to suggest that this substance be used as an index of the presence of extractive matter from fenugreek in maple products. He suggested that the periodide test recommended in his article on mayonnaise[3] be applied to an alcoholic extract of the maple product or to the hydrolyzed precipitate of choline reineckate obtained from the maple product as directed by Beattie.[4]

In view of some difficulty encountered by the writers with the periodide test, recourse was had to the microscopical study of choline reineckate which, because of its crystalline nature, lent itself to an examination by the immersion method. Although all of the optical constants could not be measured, due to the nature of the material, sufficient data were obtained to justify such a study from a determinative standpoint.

REVIEW OF THE LITERATURE

The procedure recommended by Beattie (*loc. cit.*) for precipitation of choline makes use of the reagent ammonium reineckate, $NH_4(Cr(NH_3)_2(SCN)_4)H_2O$, which unites with choline to form choline reineckate, having the formula—$(CH_3)_3N(OH)CH_2CH_2Cr(NH_3)_2(SCN)_4$.

This reagent was used by Kapfhammer and Bischoff[5] for the isolation of acetylcholine from reindeer blood. These authors transformed the acetylcholine reineckate into the gold salt of acetylcholine for identification, but as they were expecting to find choline rather than acetylcholine, they prepared choline reineckate and a gold salt of choline for comparison and published photomicrographs of the two reineckates.

H. Paal[6] states that he determined choline as a complex salt of chromium after a modification of the Kapfhammer-Bischoff method, but he does not give the procedure used nor refer to any former publication.

The Kapfhammer-Bischoff procedure is suited to the isolation of relatively large quantities of choline and was used by them to obtain about 2 grams of acetylcholine reineckate from 25 liters of reindeer blood, but it must be modified before it can be successfully applied to imitation maple flavor or sirup.

Preliminary work showed that the quantity of choline present in a well-known brand of imitation maple flavor is about 30 mg. per 100 cc. As such products are usually sold in small bottles, frequently 2 fluid

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[1] National Standard Dispensatory.
[2] *Ber.*, 38, 2518 (1885).
[3] *This Journal*, 15, 466 (1932).
[4] *Biochem J.*, 30, 1554 (1936).
[5] *Z. physiol. Chem* (Hoppe-Seyler), 191, 179 (1930).
[6] *Biochem. Z.*, 211, 245 (1929).

ounces, it was decided to aim at a procedure with which from 2 to 10 mg. of choline might be determined in a volume of approximately 25 cc. It was found that the procedure used by Beattie could be adapted to this purpose, when modified in a few particulars, including the substitution of the gravimetric for the colorimetric method of estimation of choline reineckate.

There was at hand a quantity of choline chloride believed to be pure, but it was of unknown choline content due to the hygroscopic nature of this salt. It was considered that the determination of chloride would be a reasonable basis for estimating the choline content. Accordingly, 0.321 gram of the choline chloride was weighed and made up to 100 cc. with water; 10 cc. portions were pipetted out, sodium chromate indicator was added, and the chloride was titrated with silver nitrate solution (1 cc. =1.0012 mg. of Cl). Since 1 atom of chlorine corresponds to 1 molecule of choline 1 cc. of this solution of silver nitrate=3.42 mg. of choline. Of two 10 cc. portions, one required 7.7 cc., equivalent to 26.33 mg. of choline, and the second 7.65 cc., equivalent to 26.16 mg. of choline, giving an average of 26.25 mg. of choline per 10 cc. This solution was used as a stock solution, and 10 cc. of it was diluted to 100 cc. with water for use in the experiments discussed here.

EXPERIMENTAL

As most workers with reineckate appear to have used a large excess of the reagent in the precipitation of choline, the first experiment made was to ascertain the quantity of reagent needed. The theoretical relation between choline and ammonium reineckate is 1 to 2.93. The 2.6 mg. choline contained in 10 cc. of the dilute solution would then require 7.6 mg. of ammonium reineckate.

TABLE 1.—*Excess reagent needed to precipitate choline*

CHOLINE PRESENT	REINECKATE ADDED	CHOLINE REINECKATE	CHOLINE FOUND
mg.	*mg.*	*mg.*	*mg.*
2.6	10	8.3	2.4
2.6	20	8.9	2.6
2.6	40	8.9	2.6
2.6	100	9.4	2.7

It is evident (Table 1) that 20–100 mg. of ammonium reineckate is a satisfactory quantity of reagent to be used in precipitating 2.6 mg. of choline and that it is not necessary to use 0.5 gram or more, as recommended by some workers.

Since many maple flavors contain alcohol, an experiment was conducted to ascertain the effect of alcohol upon the precipitation of choline reineckate. The results are given in Table 2.

TABLE 2.—*Effect of alcohol on precipitation of choline reineckate*

CHOLINE PRESENT	ALCOHOL BY VOLUME	CHOLINE REINECKATE	CHOLINE FOUND
mg.	*per cent*	*mg.*	*mg.*
2.6	0	8.5	2.5
2.6	10	9.0	2.6
2.6	20	8.7	2.5
2.6	30	8.9	2.6

These results (Table 2) show that the presence of alcohol up to 30 per cent by volume has no effect on the precipitation of choline in this amount.

An experiment was also conducted to establish the smallest quantity of choline that might be detected by the procedure. A quantity of choline chloride was placed in a beaker and diluted to about 30 cc.; 5 cc. of ammonium reineckate solution containing about 0.14 gram of the salt was added; and the time that intervened before a definite precipitate was visible was noted. The mixture was stirred for 2 minutes and then stood in ice water for 30 minutes, with occasional stirrings of about 30 seconds' duration. The solution was filtered and washed and the precipitate was dried and weighed as directed below. The results are given in Table 3.

TABLE 3.—*Recovery on the precipitation of small quantities of choline precipitated as reineckate*

CHOLINE PRESENT	TIME FOR PPT. TO FORM	CHOLINE REINECKATE	CHOLINE FOUND
mg.		*mg.*	*mg.*
8.4	Less than 1 min.	28.7	8.2
7.0	Less than 1 min.	24.3	7.0
6.5	Less than 1 min.	23.9	6.5
5.6	Less than 1 min.	19.3	5.5
2.8	Less than 1 min.	10.1	2.9
2.6	Less than 1 min.	9.0	2.6
1.3	Less than 1 min.	3.9	1.1
0.5	2 min.	1.2	0.3
		Color on Gooch	Color of acetone
0.5	1 min.	Pink	Pink
0.26	± 15 min.	Slight pink	Slight pink
0.13	None in 30 min.	No pink	No pink
0.05	None in 30 min.	No pink	No pink

The results in Table 3 show (1) that a visible precipitate forms in 2 minutes or less when 0.5 mg. of choline is present in 30 cc. of solution; (2) that the presence of 0.25 mg. or more of choline is indicated by a pink color on the Gooch; (3) that the acetone solution will have a noticeable pink color with 0.25 mg. or more of choline; and (4) that less than 0.25 mg. of choline is not detectable by this procedure.

A further experiment was carried out to show the effect of sugar on the precipitation of choline. In these solutions, reported in Table 4, the 30 cc. of choline solution contained 20 cc. of maple sirup.

TABLE 4.—*Precipitation of choline from solutions containing 45% of sugar*

	CHOLINE PRESENT	TIME FOR PPT. TO FORM	COLOR OF FILTER	COLOR OF ACETONE
	mg.	*minutes*		
1.	1.4	Less than 3	Pink*	Pink
2.	0.6	Less than 10	Pink†	Pink
3.	0.3	None in 30	No pink	Slight pink
4.	0.15	None in 30	No pink	No pink

* The crystals from 1 weighed 4.7 mg., equivalent to 1.4 mg. of choline.
† The crystals from 2 weighed 1.3 mg., equivalent to 0.4 mg. of choline.

These data (Table 4) show that the presence of sugar up to 45 per cent has practically no effect upon the precipitation of choline as reineckate.

In the case of many imitation maple flavors, especially those of such a strength that one teaspoonful or less is used to flavor one quart of sirup, the procedure given below will suffice to show the presence and approximate quantity of choline.

DETERMINATION OF CHOLINE AS REINECKATE

REAGENT

Place about 0.4 gram of ammonium reineckate, $NH_4(Cr(NH_3)_2(SCN)_4H_2O$,[1] in a small Erlenmeyer flask, add 15 cc. of water, shake 2–3 minutes, and filter. (The reagent must be freshly prepared just before use.)

PROCEDURE

Place 25 cc. of sample in a 50 cc. beaker and add 5 cc. of the reagent. Place the beaker in a bath of ice water, and stir 1 or 2 minutes. Allow the beaker to remain in the ice water for about 30 minutes, stirring the mixture several times for about 30 seconds each during this period.

Filter through a Gooch having a thin asbestos mat and wash the beaker and the contents of the Gooch with two or three 5 cc. portions of water, then similarly with alcohol followed by ether. Suck dry. Transfer the Gooch to another suction flask, so arranged that liquid flowing through the Gooch will be caught in a small test tube. Wash down the sides of the beaker with acetone, using 2–3 cc., and pour down the sides of the Gooch. After about 1 minute suck through any remaining acetone and repeat the operation until the acetone coming through the Gooch is no longer pink in color. If any of the pink choline reineckate dries on the outside of the Gooch, wash into the test tube with 1 or 2 cc. of acetone. Transfer the acetone solution to a weighed 10 cc. beaker and evaporate to dryness in a current of air. Wash down any crystals that may have formed on the sides of the beaker with 1 or 2 cc. of acetone and again evaporate. Place the beaker in a vacuum desiccator over sulfuric acid for several hours to remove any remaining traces of acetone. Weigh (Wt. $\times$ 0.287 = choline) and identify choline reineckate microscopically.

In the case of sirups and flavors that contain maple sugar or other

[1] Directions for preparing reineckate salt are given in Organic Syntheses, C. R. Noller, Editor, Vol. XV, p. 74.

extractive matter and owe only a part of their character to fenugreek extract, it is necessary to concentrate the choline before precipitation as reineckate. The following procedure is recommended.

CONCENTRATION

A. Solid products.—Grind the sample and mix thoroughly. Weigh 100–200 grams in a flask fitted with a reflux condenser. Add 200–250 cc. of alcohol and reflux about 1 hour. Cool, and let stand in the refrigerator overnight. Filter, and evaporate the filtrate to a small volume, adding a little water if necessary during the evaporation so that finally there will be obtained a solution in water amounting to 25–30 cc. Apply the test given under "Procedure."

B. Liquids containing 65% or more of total solids.—Place 200–500 cc. of sample in a large beaker and add 95% alcohol slowly with constant stirring until the alcohol content of the mixture reaches 80% by volume. (About 500 cc. of 95% alcohol will be required for 200 cc. of sirup.) Unless the sugar begins to crystallize at once, seed by adding a small quantity of sugar crystals, stir vigorously, and place in a refrigerator for several hours, preferably overnight.

Filter, using a Büchner funnel if the insoluble matter is crystalline (if not crystalline, decant the alcohol solution through a folded filter), and wash once or twice with 95% alcohol. Evaporate to a sirup on the steam bath; transfer to a weighed distilling flask, which should also be adapted for refluxing; and concentrate at 25–30 mm. until the solids content amounts to 70% or more. Weigh, determine solids by the refractometer, and from these data calculate the volume of water in the residue. Add slowly with stirring, 150 cc. of alcohol (or more if needed to raise the alcohol content above 80% by volume). If crystallization does not begin during the addition of the alcohol, seed as before. Reflux for 1 hour, cool, and let stand in a refrigerator overnight. Filter, and wash twice with 95% alcohol. Evaporate, repeating the precipitation of sugar if necessary, so that finally there will be obtained a solution in water amounting to 25–30 cc.; cool, and proceed as directed under "Procedure."

If a quantitative estimate of choline is desired, extract the insoluble matter obtained above twice with 95% alcohol, unite the extracts, evaporate, re-precipitate sugars with alcohol if necessary until the extract is contained in 25 cc. or less of water solution, and precipitate the choline as above.

C. Liquids containing less than 65% of total solids.—Place 200–500 cc. of sample in a weighed distilling flask that should also be adapted for refluxing. Concentrate at 25–30 mm. until the solids content amounts to 70% or more, and proceed as directed under B, beginning "Weigh, determine solids by the refractometer," etc.

MICROSCOPIC EXAMINATION

In order to identify positively the crystals obtained from commercial products, a quantity of choline reineckate crystals was prepared for microscopic examination. The procedure used follows:

A quantity (0.65 gram) of $NH_4(Cr(NH_3)_2(SCN)_4)H_2O$ was dissolved as completely as possible in 20 cc. of water and filtered into 10 cc. of water in which was dissolved 0.20 gram of $(CH_3)_3N(OH)CH_2CH_2Cl$; 15 cc. of water was used to wash out the beaker and filter used for the preparing of the reagent. A precipitate formed at once as the two solutions mingled. As the mixture was stirred the precipitate assumed a silvery pink character in a few minutes. The precipitate was filtered on a fritted glass funnel and washed once with 10 cc. of water. A solution of choline

chloride was then added dropwise to the filtrate until no more precipitate came down. The mixture was then stirred and set aside for 2 hours, when a second crop of crystals was filtered off. Both precipitates were washed with alcohol and ether and dried in a vacuum desiccator. The crystals were weighed, and nitrogen was determined by the micro-Kjeldahl method. As these crystals were very small, a portion of the material, about 0.3 gram, was dissolved in 27 cc. of acetone and allowed to recrystallize. These crystals were dried in a vacuum desiccator, and nitrogen was determined as directed previously. The recrystallized choline reineckate was then suitable for examination by the petrographic methods.

TABLE 5.—*Choline reineckate*

CROP	WT.	SAMPLE	NITROGEN*	
	gram	*mg.*	*mg.*	*per cent*
1	0.594	11.5	2.68	23.30
2	0.090	14.4	3.26	22.64
Recrystallized from acetone	0.307	13.7	3.14	22.92
Theoretical				23.21

* Determinations of N were made by B. L. Davis.

In attempting to determine the melting point of choline reineckate the analyst found that between 150° and 200° C. there was a change in color, probably due to decomposition and some sublimed material condensed outside the heated portion of the tube. At about 267° C. the substance remaining in the bottom of the tube melted to a dark brown plastic consistency. To prevent the long exposure to heat required to reach this high temperature, the melting point apparatus was heated to 255° C., and a second melting point tube containing choline reineckate was inserted. Almost immediately sublimed material appeared in the upper part of the tube, and the substance melted as before at about 268° C.

The crystals were examined microscopically by the immersion method.

OPTICAL PROPERTIES OF CHOLINE REINECKATE

In mass the material is pink, and when examined microscopically in ordinary light consists of plates, frequently with six-sided habit. These plates invariably extinguish sharply when examined with crossed nicols (parallel polarized light) on a rotating stage. The polarization colors are brilliant. When examined in convergent polarized light (crossed nicols), the material appears to be biaxial, although interference figures are rarely discernible. On this account orientation with respect to the interference figure could not be made. However, by an examination of a large number of thin, plate-like fragments oriented at random and showing maximum double refraction, it was possible to obtain refractive index values which are useful in determinative work. These are as follows: $-n_\alpha = 1.658$

(occurs frequently on the plates); n_β=not determined, and probably shown on plates tipped on edge; $n_\gamma = >1.733$ (methylene iodide); $n_i = 1.733$, an intermediate refractive index, but not the maximum value, although, in connection with the minimum value, very useful for determinative purposes. n_α and $n_i \pm 0.003$.

APPLICATION OF THE METHOD

The procedure was applied to maple sirup, as follows: 200 cc. of sirup was treated with alcohol, and the filtrate was concentrated. This concentrate was again treated with alcohol and the filtrate again concentrated. When the test was applied, no visible precipitate formed, no pink color could be seen upon the Gooch, and the acetone was colorless after passing through the Gooch.

A quart of imitation maple sirup was prepared by using 2 cups of sugar, 1 cup of water, and 4 cc. of a well-known brand of imitation maple flavor. Two samples, each containing 200 cc. of this sirup, were analyzed, and 0.6 mg. and 0.3 mg. of crystals, respectively, were obtained. In both cases the crystals were identified as choline reineckate by the refractive indices as determined by the immersion method.

It is worthy of note that the choline reineckate crystals obtained from a number of imitation maple products were found to have the odor of imitation maple. No such odor was detected on the crystals obtained from the chemically pure choline.

An extract of fenugreek seeds in 50 per cent alcohol was examined and found to contain 0.075 and 0.072 gram of choline per 100 grams of the seeds. This indicates a somewhat higher choline content than previously reported (*loc cit.*). This salt also had the maple-like odor previously mentioned in connection with the imitation maple flavors examined.

SUMMARY

A procedure is given for the quantitative precipitation and microscopic identification of choline reineckate as an index of the presence of fenugreek extract in artificially flavored maple products.

APPLICATION OF THE DITHIZONE METHOD TO THE DETERMINATION OF LEAD IN BIOLOGICAL MATERIALS

By Edwin P. Laug (Division of Pharmacology, Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

The method used by the writer for the determination of lead in biological substances is based on the procedure proposed by Wichmann *et al.*[1] Because of modifications and extensions, the method is given here in detail.

[1] Wichmann, Murray, Harris, Clifford, Loughrey, and Vorhes, *This Journal*, 17, 108 (1934); Wichmann and Clifford, *Ibid.*, 18, 315 (1935); Clifford and Wichmann, *Ibid.*, 19, 130 (1936)

Dithizone in alkaline solution reacts with at least 15 metals; however, in the presence of citrate and cyanide, the specificity is increased and only lead, bismuth, stannous tin, and thallium combine with this reagent. Stannous tin did not interfere, and thallium and bismuth rarely interfered in the procedure outlined. Less than 1 gamma of lead can easily be determined by this method, therefore all reagents must be practically free of lead.

REAGENTS

(1) *Water.*—Since the available distilled water was higher in lead than the tap water, the latter was slightly acidified and distilled in an all-glass Pyrex apparatus fitted with a double trap to prevent spray. The distillate contained approximately 0.6 gamma of lead per liter.

(2) *Chloroform.*—Fresh $CHCl_3$, redistilled in an all-glass apparatus.

(3) *Dithizone.*—Repurified by the method of Wichmann *et al.*[1]

(4) *Ammonium hydroxide.*—Reagent grade, redistilled in an all-glass apparatus into ice-cold distilled water. Approximately 14 *N*.

(5) *Nitric acid.*—Reagent grade, redistilled.

(6) *Hydrochloric acid.*—Reagent grade diluted 1:1 and redistilled. Approximately 7 *N*.

(7) *Ammonium citrate.*—Crystalline citric acid dissolved in concentrated NH_4OH, with cooling. The reagent was a 50% (W/V) solution. Lead as an impurity was removed from the strongly alkaline mixture by repeated extractions with a $CHCl_3$ solution of dithizone. Residual dithizone dissolved in the aqueous phase, forming an orange-colored solution, must be removed by washing with $CHCl_3$ until water clear to prevent subsequent interference.

(8) *Cyanide.*—Commercial grades contain lead and probably also phosphates as impurities, and the phosphates are a serious source of interference due to the formation of insoluble lead phosphate. Purification was effected by adding 1 or 2 cc. of a concentrated solution of $CaCl_2$ to a 45% (W/V) solution of NaCN. The flocculent precipitate carried down the lead and presumably also calcium phosphates. After settling overnight, the solution was filtered through a sintered glass filter.

From the purified reagents, the special reagents were prepared as follows:

(a) *Dithizone in chloroform.*—See concentrations in Table 1.

(b) *Nitric acid, 1 per cent.*—10 cc. of Reagent (5) diluted to 1 liter with Reagent (1).

(c) *Citrate-cyanide-ammonia reagent, concentrated.*—53 cc. of Reagent (8) and 180 cc. of Reagent (4) were made to 2 liters with Reagent (7).

(d) *Citrate-cyanide-ammonia reagent, dilute.*—53 cc. of Reagent (8), 180 cc. of Reagent (4), and 200 cc. of Reagent (7) were made to 2 liters with Reagent (1).

PREPARATION OF SAMPLE

Dry the tissues to constant weight, place either in small Pyrex beakers or glazed porcelain casseroles, and thoroughly char in a gas oven or on a hot plate for 2–3 hours. Then cover with watch-glasses and place the containers in an electric muffle furnace at 500° C. for 18–24 hours. (This procedure usually removed most of the carbon, leaving a gray ash.) Destroy any residual carbon by "regenerating" with 2–3 cc. of concentrated HNO_3, evaporating just to dryness, and returning the containers to the furnace for 20 minutes. Dissolve the resultant white ash by warming with 2–4 cc. of 7 *N* HCl, transfer to a volumetric flask, and make to volume with distilled water. (Water-clear solutions result in most cases.)

[1] *Loc. cit.*

ISOLATION OF LEAD (1–10 GAMMA)

Measure 50 cc. of Reagent (b) into a 250 cc. Pyrex separatory funnel, and add the solution containing the known or unknown quantity of lead. Make this mixture alkaline by slowly adding 10 cc. of Reagent (c), which usually adjusts the pH to about 9. However, if the solution is still acid, adjust the pH by adding Reagent (4). Extract lead by shaking vigorously one hundred times with 10 cc. of a 50 mg. per liter solution of dithizone in $CHCl_3$. (The extent to which some of the excess dithizone enters the aqueous phase, coloring it bright orange, is conditioned by the alkalinity. This serves as a convenient indicator for determining whether the pH is optimum for complete extraction of the lead.) If, after shaking, no pronounced color results, add more NH_4OH. Draw off the $CHCl_3$ layer containing the lead-dithizone complex, except for 1 or 2 drops, into a second funnel containing 50 cc. of Reagent (b). Wash out the globules of the $CHCl_3$ solution of the lead-dithizone complex remaining behind in the first funnel by shaking the solution with 10 cc. of $CHCl_3$ and drawing this off into the second funnel. (Experiment has shown that no lead remains in the aqueous solution in the first funnel.) Now vigorously shake the second funnel one hundred times to decompose the lead-dithizone complex and take the lead into the HNO_3 as the nitrate. After separation of the two phases, draw off and discard the "spent" dithizone solution, except for a few drops. (Tests have shown that no lead is lost when the $CHCl_3$ solution is rejected.) Wash the aqueous phase by shaking vigorously a few times with 5 cc. of $CHCl_3$. After it has been allowed to stand for about 5 minutes, with occasional agitation to dislodge any $CHCl_3$ at the surface of the aqueous phase, very carefully draw off until as little as possible remains in the funnel. (This procedure is extremely important, because if some droplets remain behind they will dilute the final colorimetric reagent. At best, of course, some $CHCl_3$ remains in the bore of the stopcock.) To prevent this remaining $CHCl_3$ from diluting the lead-dithizone solution when this is drawn off for the colorimetric determination, discard the first few drops running through the stopcock. Make the 50 cc. of 1% HNO_3, freed as completely as possible from $CHCl_3$, and containing the lead, alkaline by the addition of exactly 10 cc. of Reagent (d). (The pH of the solution is buffered at about 9.5.) Add exactly 10 cc. of a $CHCl_3$ solution containing 6.5 mg. of dithizone per liter, and shake the funnel vigorously one hundred times. After separation, draw off the $CHCl_3$ layer through a cotton plug in the delivery tube of the funnel to remove any small quantities of water emulsified in the $CHCl_3$. Place the colored solution in a 15 cc. Pyrex test tube, tightly stopper with a cork stopper, and store in a cool, dark place until a reading can be made in the spectrophotometer.[1] (No detectable fading occurs under these circumstances within 24 hours.)

Read the absorption of light at a wave length of 510 mμ on the density scale of

TABLE 1.—*Ranges for the determination of lead in spectrophotometer*

LEAD RANGE	TUBE LENGTH	TUBE VOLUME	SOLUTION OF DITHIZONE: VOLUME: PRELIMINARY EXTRACTION	VOLUME: FINAL EXTRACTION	CONCENTRATION: PRELIMINARY EXTRACTION	CONCENTRATION: FINAL EXTRACTION	DENSITY READING RANGE	DENSITY READING PER GAMMA OF LEAD
gamma	*cm.*	*cc.*	*cc.*	*cc.*	*mg./liter*	*mg./liter*		
0– 5	10.0	9.5	10	10	50	6.5	0 90–2 00	0 25 –0.27
0– 10	5 0	9.5	10	10	50	6.5	0.50–2.00	0.13 –0.14
0 100	1.0	2 0	25	20	100	25.0	0.50–2.00	0 0125–0.013

[1] The neutral wedge photometer developed by Clifford and Wichmann, *loc. cit*, is a very satisfactory substitute for the spectrophotometer.

the spectrophotometer and relate it to lead content by a straight-line function constructed with suitable lead standards.

Determinations of lead in amounts from 0 to 100 gamma were conveniently made by using all-glass tubes 10, 5, and 1 cm. in length. The data are presented in Table 1. Volumes and concentrations of dithizone for the colorimetric extraction were adjusted to cover the useful range only (0–2.00) of the density scale. In Fig. 1 are

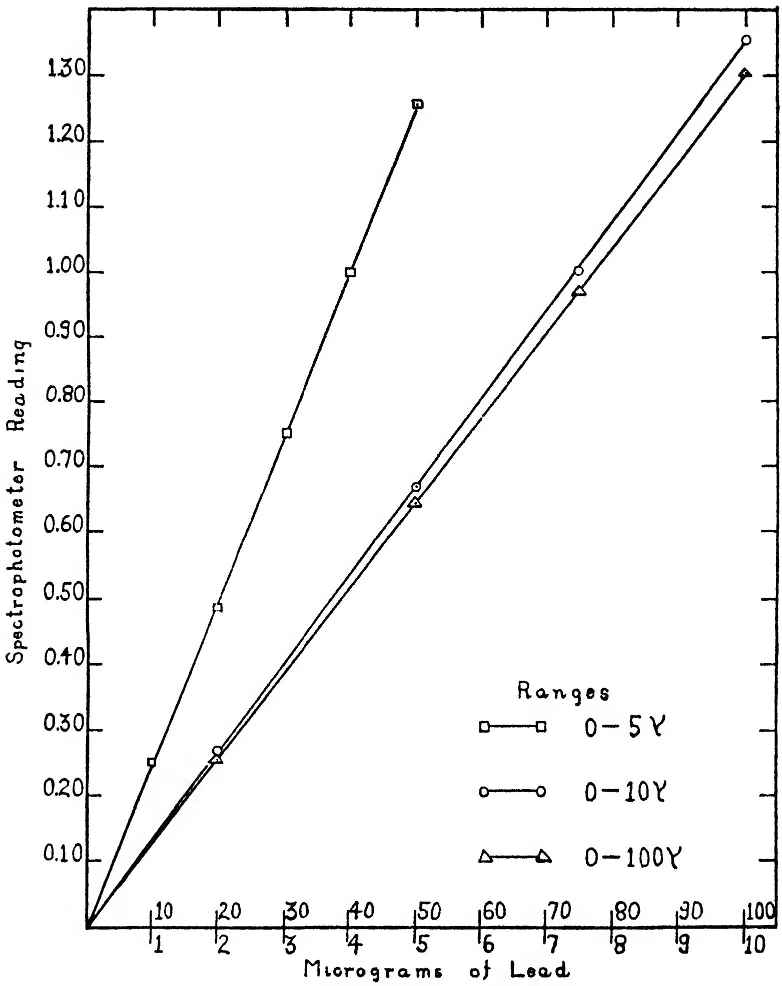

FIG. 1.—THE RELATION BETWEEN LEAD CONCENTRATION AND SPECTROPHOTOMETER READING

shown typical calibration curves, made with known quantities of lead in the three ranges. Routinely, with every set of unknown determinations, from 2 to 5 points were made with standard lead solutions. This was necessary for two reasons. First, small fluctuations were observed of the order of 1–2 per cent, which were attributable to variations in room temperature. Thus, it was found that in the range 0–10 gamma, there occurred differences in slope of 10 per cent (for winter it averaged

12.8 at 20°–25° C., and for summer, 13.3 at 28°–32° C.). Second, it was found that the standard dithizone solution gradually fades on standing. While the absorption of light at 510 mμ by the dithizone is minimum, it is nevertheless measurable and will reflect changes in the concentration.

DISCUSSION

Interference by phosphates.—The most serious interference with the accurate determination of lead by the method presented here was the presence of phosphates. While citrate will render the phosphates soluble within certain limits, excessive amounts cannot be tolerated, because of the formation of a flocculent precipitate which carries with it practically all the lead in a form no longer extractable with dithizone. When a mere trace of precipitate has formed after the solution has been made alkaline, it has been the practice to discard the sample and use a smaller aliquot. This type of interference, however, is not always heralded by the formation of a precipitate. Under certain circumstances, in the absence of a large amount of calcium, colloidal and invisible lead phosphate may form.

In Table 2 the results of the analysis of 10, 20 and 30 cc. aliquots of a 250 cc. solution of a rat carcass are presented. Up to 20 cc. no phosphate interference occurred, as shown by the fact that exactly twice (and not less) as much lead was recovered from the 20 cc. aliquot as from the 10 cc. aliquot. Complete recovery of added lead confirmed this finding. When a 30 cc. aliquot was taken, visible precipitation occurred, and no lead was found. Even when lead was added, only a small proportion of it was recovered. In view of this experience, it became the practice in many cases to determine lead on two aliquots of different sizes. The presence of large amounts of phosphates was thus discovered and many errors were avoided.

TABLE 2.—*Recovery of lead added to different aliquots of a solution of rat carcass*

ALIQUOT	LEAD ADDED	TOTAL LEAD FOUND	LEAD RECOVERED	
cc.	*gamma*	*gamma*	*gamma*	*per cent*
10	0	1.4	—	—
10	0	1.4	—	—
10	5.0	6 4	5 0	100
10	5.0	6.4	5.0	100
20	0	2 8	—	—
20	0	2.8	—	—
20	5.0	7.7	4.9	98
20	5.0	7.8	5.0	100
30	0	0	—	—
30	5.0	1.1	1.1	20

Stannous tin.—By the method outlined, stannous tin would be extracted by the dithizone and reported as lead. Fortunately, however, in the process of ashing and regeneration with nitric acid, any stannous tin

was oxidized to the stannic form. In Table 3 are presented the results of treating known amounts of stannous tin in the presence and absence of lead with concentrated nitric acid by heating in a casserole. It is clearly demonstrated that tin in the concentrations used in this study does not interfere with the determination of lead by this procedure. The tin is converted to insoluble metastannic acid, which itself is neither extracted, nor does it prevent the extraction of lead. When, however, 20 gamma of stannous tin was extracted with dithizone directly without preliminary oxidation, a reading was obtained equivalent to 10 gamma of lead.

TABLE 3.—*Extent of stannous tin interference with lead method*

STANNOUS TIN ADDED TO CASSEROLE AND OXIDIZED WITH NITRIC ACID	LEAD ADDED TO CASSEROLE	LEAD RECOVERED
gamma	*gamma*	*gamma*
250	0	0
250	5	5
0	5	5
1250	5	5
1250	0	0
0	5	5
20*	0	"10"

* Not oxidised with HNO_3 before determination

It has been stated that while oxidation of stannous to stannic tin may be easily effected by nitric acid, subsequent reduction to stannous tin by extraction with dithizone may occur. This was not the experience of the writer, however, for repeated extraction of lead in the presence of oxidized tin salts showed that in the quantities used here the tin did not interfere with the lead determination.

Keeping qualities of pure dithizone and lead-dithizone solutions.—Provided the dithizone was kept in the dark and well stoppered to prevent concentration changes due to loss of chloroform, it deteriorated very slowly. The readings made at intervals on pure and partially lead-

TABLE 4.—*Keeping qualities of pure dithizone and lead-dithizone solutions kept dark in glass-stoppered flasks*

DATE READ	DENSITY READINGS ON SPECTROPHOTOMETER					
	PURE DITHIZONE		DITHIZONE+5 GAMMA LEAD		DITHIZONE+10 GAMMA LEAD	
10/27/36	0.61	0.61	1.23	1.22	1.87	1.87
10/30/36	0.61	0.61	1.21	1.22	1.85	1.85
11/10/36	0.55	0.57	1.17	1.19	1.85	1.85
12/19/36	0.52	0.55			1.83	1.85

saturated dithizone solutions for a period of almost two months are given in Table 4. It will be noted that there is a slow loss of tinctorial power. The largest decrease occurred in the pure solution, while the partially lead-saturated solutions showed small changes, which is in keeping with the oft-repeated observation that the colored lead complex is more stable.

In contrast, exposure of the dithizone solutions to light for relatively short periods produced marked visible fading. Sixty hours' exposure of a pure dithizone solution reduced the average reading of 3 samples from 0.330 to 0.300; 18 hours' exposure of two other solutions showed a reduction from 0.350 to 0.300. Two dithizone solutions containing lead, exposed to light for 60 hours, gave a reading of 1.54 as against an initial reading of 1.55, a change of only 0.01.

Ash-aid.—Satisfactory recoveries and determinations were obtained with the types of material used in this investigation without the use of ash-aid. In materials containing a very low ash, such as candies and jellies, it was found desirable to use ash-aid. This was also found to be true if the ashing was carried out at temperatures above 500° C.[1] When ash-aid is used, or when materials high in inorganic salts, such as bone, are being used, it must be strongly emphasized that the hydrochloric acid procedure advocated by Clifford and Wichmann (*loc. cit.*, 3, page 153) be exactly followed so that a crystal-clear solution be obtained or, as the writer found, losses will occur.

SUMMARY

Details necessary for the successful application of the dithizone method to the determination of lead in biological material have been presented. They may be summarized as follows:

(1) Phosphates must be removed almost completely in order to prevent precipitation of lead in a form not amenable to dithizone extraction.

(2) Interference by small quantities of stannous tin was prevented by preliminary oxidation to the stannic condition. No interference due to reduction of stannic to stannous tin by dithizone was observed.

(3) Dithizone and lead-dithizone solutions in chloroform were found to be quite stable when stored in the dark, but they decomposed readily under the influence of light.

(4) The dithizone method gave accurate results for direct analysis of biological materials containing lead ranging in quantity from less than one to 100 gamma.

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[1] Wichmann and Clifford, *loc. cit.*

THE EVALUATION OF YELLOW MUSTARD*

By ARNO VIEHOEVER and WALTER L. NELSON† (Gross Laboratory for Biological and Biochemical Research, Philadelphia College of Pharmacy and Science, Philadelphia, Pa.)

Sinapis alba L. (white or yellow mustard) is a valuable spice and medicinal agent known since ancient times. Thus far, however, no method of evaluation has been worked out, probably because its composition is peculiarly different from that of the black or brown mustards. These yield, upon enzymatic fermentation, a volatile oil that can be quantitatively determined (1, 2), while white or yellow mustards yield a non-volatile oil, which, extracted together with the fatty oil, has not been isolated quantitatively. A method is herein described for the quantitative isolation of the glucoside sinalbin and for the estimation of the approximate amount of pure sinalbin present by means of its sulfur decomposition product obtained by the action of the enzyme myrosin.

I. CHEMICAL COMPOSITION OF MUSTARDS

The chart shows the chemical composition of white mustard, contrasted to the composition of the black. The fermentative decomposition products are also mentioned. It is quite obvious that the black mustard, and es-

Comparison of the chemical composition of Sinapis Alba and Nigra

GLUCOSIDE	ENZYME	DECOMPOSITION PRODUCTS OF GLUCOSIDE ENZYME			
		MUSTARD OIL	SUGAR	SALT	ALKALOID
		Sinapis Nigra			
Sinigrin 1.3% (3)	Myrosin Saccharase Amylase Maltase Emulsin Peroxydase	Allylisothio-cyanate 96–97.77% Small Amount allylcyanide, Propenyl iso-thiocyanate Allylrhodanide Carbon disulfide	Dextrose	Potassium bi-sulfate	Sinapin
		Sinapis Alba			
Sinalbin 2.5% (4)	Myrosin	Para-Oxyben zylisothio-cyanate 0.16% (4) (6)		Dextrose	Sinapin bisulfate
Sinigrin (5)					

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† Working with a grant of the Kilmer Research Fellowship.

Comparison of the chemical composition of Sinapis Alba and Nigra (Continued)

DECOMPOSITION PRODUCTS	FATTY OILS	PHYTIN-ACID	OTHER CONSTITUENTS
	Sinapis Nigra		
Sinapin acid and Cholin	28.3% (7) Sp. gr. 0.9212 at 15° C. Solid. pt. −15° to −16° Ref. Ind. 1.4739 at 20° Acid No. 2.6 Calc'd. as free oleic acid 1 02% Sap. No. 173.2 Ester No. 170.6 Iod. No. (Wij's) 101.1 Glycerol 9 32 Unsaponifiable 1.18% Fatty acids 24.16% (94.16% Viehoever?)	Anhydro-hexamethylene di-Phosphoric acid	Protein 29.11% N.-Free Extr. 19.23% Crude fiber 10.9% Ash, 4 9% Water, 7.5% Pigment (8) Fat 28.1%
	Sinapis Alba		
	28.1% (7) Sp. gr. 0.9214 at 15° C. Solid pt. −15° to −16° Ref. Ind. 1.4704 at 20° Acid No. 2.6 Calc'd. as free oleic acid 1.32% Sap. No. 177.8 Ester No. 175.2 Ind. No. (Wij's) 96.8 Glycerol 9 57% Unsaponifiable 0.98% Fatty acids 94.23%		Protein 27.6% Fat 29.7% N-Free Extr. Sub. 20.8% Crude Fiber 10 3% Ash. 4.47% Water 7.2% Pigment (8)

pecially the products formed as a result of the enzymatic action, have been studied more thoroughly. Quite probably enzymes other than myrosin, as found in black, occur also in yellow mustard, and, in consequence during their fermentative action, products may be formed, not as yet reported. The possibility also exists that the enzymatic hydrolysis with myrosin yields small amounts of other substances, not as yet identified. The results of the experiments with myrosin, related later in this paper, suggest such a thought.

II. ISOLATION OF THE GLUCOSIDE SINALBIN

The sinalbin used was prepared by the following method: 100 grams of fresh yellow mustard flour was defatted with 1200 cc. of petroleum ether, successive portions of 200 cc. each being used. The defatted flour was

thoroughly dried and extracted in the earlier experiments with boiling 85 per cent ethyl alcohol. In later experiments the defatted flour of ground mustard was boiled on the steam bath with 5–6 times the amount of 95 per cent ethyl alcohol used in three successive extractions. These were filtered as hot as possible through a Büchner funnel and then set aside in the refrigerator. The crystals separated singly on the glass wall, in the liquid, and on the bottom as white or whitish crystal aggregates. The alcoholic extract was allowed to stand overnight at room temperature. The sinalbin crystallized out in slightly yellow, needle-like crystals, which were further purified by recrystallization from boiling 85 per cent ethyl alcohol and washing with acetone. Sinalbin that has been recrystallized and thoroughly washed will still show the presence of a yellow coloration when dissolved. Will and Laubenheimer (4) had already observed that sinalbin becomes yellow, forming sinapin compounds in the presence of traces of alkali, as present even in tap water.

The sinalbin, when estimated by extracting with several portions of alcohol and proceeding as above, showed a content in freshly prepared yellow mustard flour of about 2.5 per cent. The yield of crude sinalbin in a 200 gram sample was 5 grams; in a 100 gram sample it was 2.82 grams and in a 50 gram sample it was 1.35 grams. The complete extraction of sinalbin was readily verified by the absence of a color reaction upon testing the final warm extracts with Millon's reagent, as they gave a red coloration with sinalbin according to Hartwich and Vuillemin (9).

A sample of sinalbin, free from yellow coloration and from ash and showing marked needle structure (with striking colors under the polarizing microscope) was prepared by the following method. The slightly yellow crystals of sinalbin were dissolved in boiling 85 per cent ethyl alcohol and allowed to cool to approximately 60° C. Ether was slowly added to this solution until the yellow color disappeared and a white precipitate was formed. (Avoid using a large excess of ether as then a heavy amorphous precipitate is formed.) This precipitate was dissolved in boiling 95 per cent ethyl alcohol and filtered while still hot, and the sinalbin was crystallized from the filtrate. The sample had a melting point of 100–102° C. The melting point determination was repeated with various lots and found to be practically the same. (Gadamer (3) reported a melting point of 83–84° C. for the air-dry product, a loss of crystal water at 100° C. with partial transformation and partial fusion, complete fusion at 138–140° C.)

III. ISOLATION OF THE ENZYME MYROSIN

The myrosin solution used was prepared as follows: Fifty grams of yellow mustard flour was extracted with 150 cc. of distilled water, an equal volume of 95 per cent ethyl alcohol was added, and the mixture was centrifuged. The alcohol-water mixture was decanted off and the

	$BaSO_4$	SINALBIN CALC'D FROM $BaSO_4$	PURITY
	gram	*grams*	*per cent*
Series I			
A. Myrosin solution—100 cc. Sinalbin (recrystallized)—1.3856 grams	.3964	1.246	89.92
B. Myrosin solution—100 cc. Sinalbin (recrystallized)—1.4826 grams	.4236	1.332	89.84
Series II			
A. Myrosin solution—50 cc. Sinalbin (not purified)—.5023 grams	.1320	.4151	82.62
B. Myrosin solution—50 cc. Sinalbin (not purified) X—.5123 gram	.1340	.4214	82.25
Series III			
A. Myrosin solution—50 cc. Sinalbin (ether purified)—.2890 gram	.0820	.2578	89.20
B. Myrosin solution—50 cc. Sinalbin (ether purified)—.2072 gram	.0589	.1846	89.09
Series IV			
A. Myrosin solution—20 cc. Sinalbin (not purified)—.0774 gram	.0197	.0620	80.08
Series V			
A. Myrosin solution—20 cc. Sinalbin (recrystallized once)—.0162 gram	.0162	.0141	86.83
Series VI			
A. Myrosin solution—30 cc. Sinalbin (acid purified)—0.2 gram No sharp M.P. sublimes between 140–145° C.	.0488	.1534	76.7
Series VII			
A. Myrosin solution—30 cc. Sinalbin (alcohol purified)—0.2 gram M.P. 105–107° C.	.0604	.1856	92.8
B. Myrosin solution—?0 cc. Sinalbin (alcohol purified)—0.1235 gram M.P. 105–107°C.	.0363	.1141	92.4
Series VIII			
A. Myrosin solution—20 cc. Sinalbin (filtered and precipitated from alcohol solution)—0.0966 gram	.0293	.0921	95.4
Series IX			
A. Sinalbin (recrystallized)—0.0577 gram M.P. 98° C.	.0171	.0539	93.4
Series X			
A. Sinalbin (recrystallized)—0.0384 gram M.P. 102–103° C.	.0122	.0383	99.96

residue washed several times with 70 per cent ethyl alcohol. After thorough washing the residue was mixed with 50 cc. of distilled water and allowed to stand for 12 hours at room temperature. It was filtered after standing, and barium chloride (20 cc. of a 10 per cent solution) was added and the mixture set aside in an ice chest for 3 days. The solution was filtered, and a 10 cc. portion was boiled with 2 cc. of concentrated hydrochloric acid. No precipitation of barium sulfate was noted upon the addition of barium chloride. The filtered, clear, myrosin solution was used for the sinalbin-myrosin reaction.

IV. THE ACTIVITY OF MYROSIN UPON SINALBIN

The reaction between sinalbin and myrosin was carried out in tightly fitting glass-stoppered bottles. Weighed amounts of sinalbin were added to the aged myrosin solution and agitated to insure solution (10). Toluene (1% of the liquid volume) was added, and the mixture was set aside at room temperature for 24 hours. This solution was then boiled with 3 cc. of concentrated hydrochloric acid, and barium chloride (20 cc. of 10 per cent solution) was added. The precipitated barium sulfate was filtered into a weighted Gooch crucible, dried, and weighed as barium sulfate. The reaction between sinalbin and myrosin is assumed to proceed as follows:

$$\underset{\text{Sinalbin}}{C_{30}H_{42}O_{15}N_2S_2} + \text{Myrosin} + H_2O = \underset{\text{Dextrose}}{C_6H_{12}O_6}$$
$$+ \underset{\substack{\text{Para hydroxy-}\\ \text{benzyl-iso-}\\ \text{thiocyanate}}}{C_7H_7O \cdot N\ CS} + \underset{\substack{\text{Sinapin acid}\\ \text{sulfate}}}{C_{16}H_{24}O_5N \cdot H\ SO_4}.$$

The general procedure followed was that of Neuberg and Wagner (10), who studied the enzymatic effect of myrosin on sinigrin.

The molecular ratio is: 1 Ba SO_4 = 1 sinalbin. The following general equation was used for calculating the purity of sinalbin:

$$\frac{\underset{\text{of Sinalbin}}{\text{Mol. Wt.}} \times \underset{\text{found}}{\text{Wt. of } BaSO_4} \times \underset{100}{\text{Per cent}}}{\text{Mol. Wt. of } BaSO_4 \times \text{Wt. of Sinalbin used}} = \%\ \text{of Sinalbin.}$$

The preceding table lists the results obtained on samples of sinalbin.

CONCLUSIONS

1. Estimation of the glucoside sinalbin in yellow mustard flour by extraction with boiling ethyl alcohol showed a content of sinalbin of approximately 2.5 per cent.

2. The purity of the samples, without recrystallization, determined

by its decomposition products with myrosin, gave results of 82–83 per cent sinalbin.

3. The determination of sinalbin on recrystallized samples shows a purity varying from 89.09–99.96 per cent, which indicates the possibility of evaluating yellow mustard by establishing a check on the amount of the isolated glucoside.

4. The recrystallized samples of sinalbin were ash free, gave water-clear solutions in alcohol (hot) and cold water, and a melting point of 100–102° C., as compared with the literature reports of 84° C. for the air-dried, and 138–140° C. for the oven-dried sinalbin.

5. It is reasonable to assume that the enzymatic hydrolysis does not always go to completion, or that side, if not reversible, reactions might take place, as they evidently do in the enzymatic breakdown of sinigrin, the glucoside of black mustard.

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THE SENSITIZATION OF PAPER STRIPS WITH FILTERED MERCURIC BROMIDE SOLUTION IN THE GUTZEIT METHOD OF ARSENIC ANALYSIS*

By Richard S. Rosenfels† (California Agricultural Experiment Station, Davis, Calif.)

During work at this Station on arsenic fixation by soils by Rosenfels and Crafts,[1] it was observed that the mercuric bromide solution used to sensitize Gutzeit strips need not be freshly prepared if it is filtered prior to use.

Standard strips were prepared according to the directions given in the third (1930) edition of the A.O.A.C. book of methods[2] except that the generator bottles were immersed in an ice bath for 10 minutes prior to the addition of the zinc. After the zinc had been added the evolution of

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[1] *Hilgardia*, in press.
[2] *Methods of Analysis, A.O.A.C.*, 1930, 306.

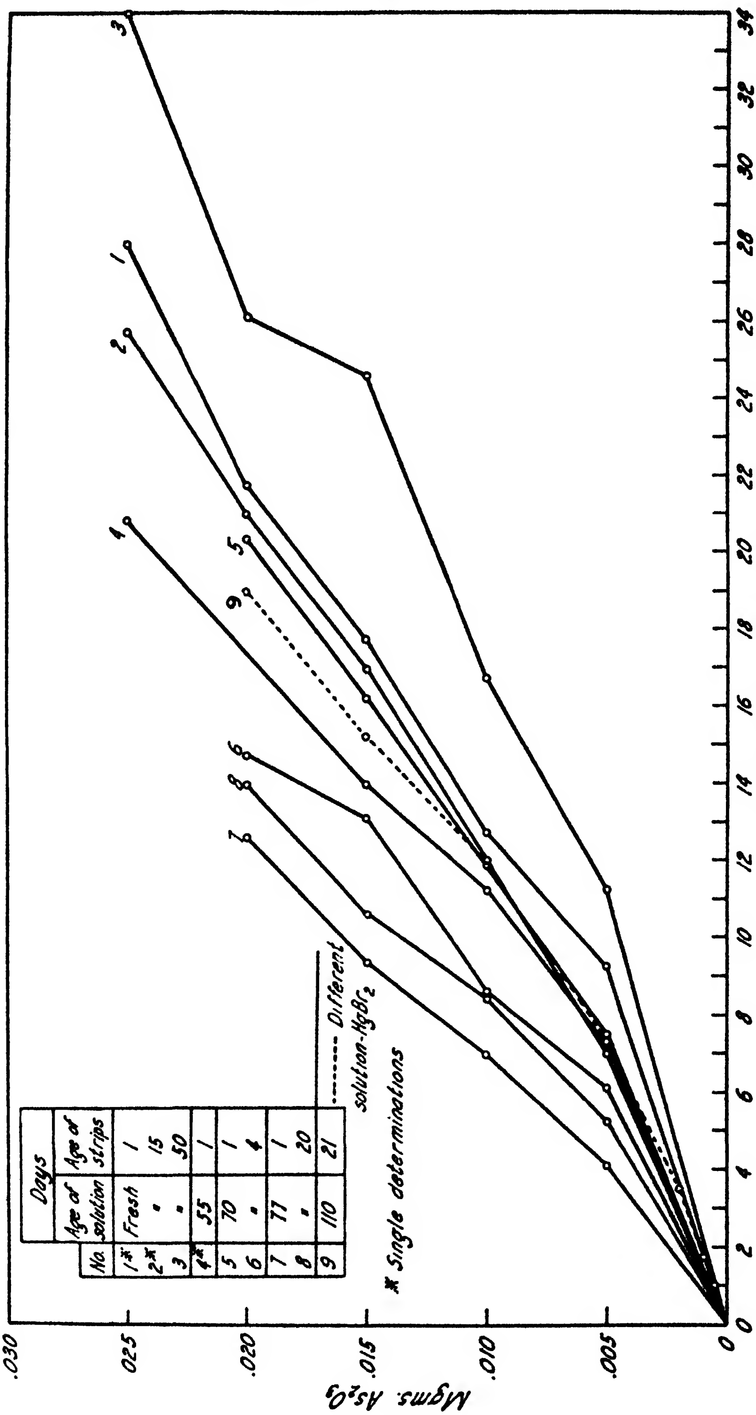

FIG. 1.—LENGTH OF STAIN IN RELATION TO AGE OF FRESHLY FILTERED MERCURIC BROMIDE SENSITIZING SOLUTION AND AGE OF STRIPS

arsine was allowed to proceed for 20 minutes in the ice bath, and then for 1 hour at room temperature. Approximately 3 grams of 20-mesh granular zinc was used.

Figure 1 shows the relation between amount of arsenic and length of stain for nine sets of standard strips. Except where indicated, all determinations were in duplicate, and each strip was measured on both sides, each point on the curves thus representing the mean of four measurements of length of stain.

Curves 1 to 8, inclusive, represent standard strips made from the same mercuric bromide solution. Curve 9 (dotted line) represents a different solution. However, all lines represent 4 per cent mercuric bromide in 95 per cent ethyl alcohol. The Hanford-Pratt sheet was soaked in the solution 1 hour, dried by waving in the air, and stored between sheets of filter paper to prevent curling. Unfortunately Cassil's observation[1] that better strips can be obtained by soaking under reduced pressure was not received until the work here described was completed. The mercuric bromide solution, when not freshly prepared, was filtered through a Schleicher and Schüll folded filter, No. 588, just before use in each instance recorded in Fig. 1.

Judged from Fig. 1, the mercuric bromide solution need not be freshly prepared. Thus, Curve 3 was obtained from strips run 50 days after sensitization. The end points were very indistinct, and the lengths of stain extreme. The original mercuric bromide solution was filtered shortly after this (55 days after it was originally prepared), and Sheet 4 sensitized. As shown by Curve 4, obtained the next day, the stain lengths for each amount of arsenic were markedly reduced. Also, the end points were sharp, and the results were fully as satisfactory as those obtained with Curve 1, which resulted from a freshly prepared solution. The original mercuric bromide solution was filtered twice again, at 70 and 77 days after it was first prepared. Each filtration served to remove any precipitate that might have formed, and the strips produced were entirely satisfactory. The most extreme case recorded is that of Curve 9, wherein the solution was 110 days old when filtered and used, and the strips were not used for 21 days thereafter.

As the fact last mentioned suggests, the strips apparently need not be freshly sensitized. Although strips 50 days old were unsatisfactory, Curves 2, 8, and 9 represent strips 15, 20, and 21 days old, respectively, and in each case satisfactory results were obtained. Comparisons of 1 and 2, and of 7 and 8, reveal no marked changes occurring during the 15–20 days that elapsed.

The length of stain decreased markedly in the 3 days that elapsed between Curves 5 and 6, a fact emphasizing the desirability of preparing a set of standard strips with each set of unknowns.

[1] *This Journal*, 20, 171 (1937).

To summarize, the filtration technic permits the use of a relatively large quantity of mercuric bromide solution for sensitization without the necessity for discarding it soon after it is first used. The solution can be filtered and used repeatedly for at least 3 or 4 months at a considerable saving of time and materials.

DETERMINATION OF EGG QUALITY BY A SAMPLING METHOD*

By SAM R. HOOVER (Food Research Division, Bureau of Chemistry and Soils, Washington, D. C.)

The evaluation of "quality" in shell eggs is based upon the measurement of certain physical and chemical characteristics. The most obvious of these is the proportion of thin to thick white. The methods of observation fall naturally into two groups: those in which the egg is broken out and the contents examined, and those in which observations are made through the shell. At present the only procedure of the latter sort in general use is candling.

The principle involved is that the yolk, visible through the shell before the candle, and surrounded by its bag of thick white, rises to the top of the egg through the thin white, but does not travel through the thick white. Thus, the greater the quantity of thick white around the yolk, the greater the distance remaining between it and the surface of the egg's liquid contents.

Experience has shown that eggs may be evaluated in this manner with considerable certainty, especially when the eggs are of low grade. The elimination of eggs containing blood spots, rots, etc., is probably the most important function of candling, as the smaller differences between good eggs and the best eggs are less easily seen. Candlers judge an egg by the size of the air cell, which according to Sharp[1] bears little relationship to interior quality. Furthermore, eggs with unusually dark shells or dark yolks are less translucent, and may be placed in lower grades than is actually warranted. In general, the criteria used in candling have not, so far as the writer knows, been successfully evaluated in terms of the quality of the broken-out egg.[2] This point is of great importance in establishing the differences between standards, extras, and specials. It is therefore desirable to have other methods to supplement the candling, even if their use involves a sampling process and the breaking of the eggs examined.

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* Food Research Division Contribution No. 390.

[1] *Food Research*, 2, 477 (1937).

[2] Van Wagenen and Wilgus obtained a significant correlation between yolk visibility, yolk mobility, candlers grade, and the photographic score of interior quality. This was done with eggs candled especially for those factors. In commercially candled eggs Parsons and Mink observe a correlation between score and candled grade, but with wide overlapping. See also Almquist, California Agr. Exp. Sta. Bull. No. 561 (1933), and Sharp, *loc. cit.*, for discussion of the value of candling as a measure of broken-out quality.

Workers in this field have proposed various physical measurements of the yolk and white, all of which have value. Seven of these methods have been compared by Parsons and Mink,[1] the standard of judgment being the photographic method of Van Wagenen and Wilgus.[2] The quickness with which the broken-out eggs may be compared to standard photographs recommends this technic, and it represents rather well the quality as judged by the housewife. However, its subjectivity is apparent, and a more objective method of checking egg grading is needed.

A closer consideration of the intrinsic qualities that influence those judging broken-out eggs reveals that "the two chief factors in this observation are the outline of the firm albumen viewed from the top, and the outline viewed from the side."[2] The grade becomes lower as the height of thick white decreases, and its area increases. The average figure for the height of the thick fraction in strictly fresh eggs is 6.5 mm. for an area of about 50 sq. cm. It is probable, however, that the area of the thick white is by far the more important factor affecting this judgment of quality.

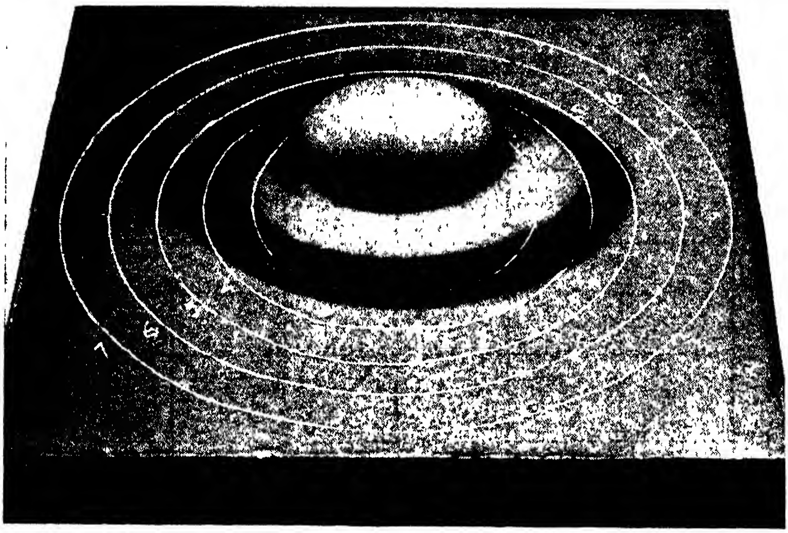

FIG. 1.—EGG ON THE AREA MEASURING PLATE

Accordingly, Parsons and Mink evolved a new method, the albumen area index, which is the ratio of the area of the thick white to its weight. The area is obtained by tracing the outline of the thick white on cellophane held on an inverted petri dish over the broken egg, and measuring the figure with a planimeter. The amount of thick white is obtained by weighing that fraction of the white held back by the conventional screen.

[1] *U. S. Egg Poultry Mag.*, 43, 484 (1937).
[2] *J. Agr. Research*, 51, 1129 (1935).

The correlation of quality determined from the photographic score with the several physical methods made by Parsons and Mink shows a fairly good agreement (1) for mean values of the albumen index (mean height/mean width), or (2) for mean values of the albumen area index (area of thick white/weight of thick white), and (3) for value of the area of thick white. However, the variations within a group are large, as is shown by the spread of the results.

Of these three measurements, the area of thick white is the only one

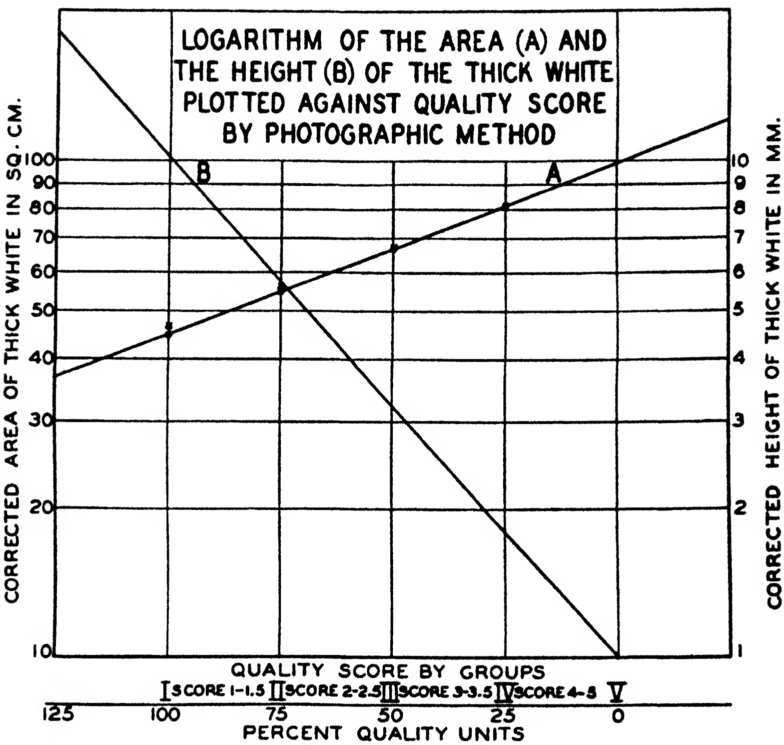

FIG. 2

SMALL DOTS DENOTE DATA OF PARSONS AND MINK, MEAN AREA WITHOUT WEIGHT CORRECTION. X DENOTES WRITER'S DATA, MEAN CORRECTED AREA

that does not include a correction for the size of the egg; the other two are respectively, ratios of height to width and of area to weight of the ellipsoidal spot of thick white. Therefore the spread of the results in the case of the area would certainly be still less if it were corrected for the weight of the egg.

In another recent paper on this subject Haugh[1] found that the photographic quality score varies as the negative logarithm of the height of the thick white. Haugh considers that this relationship is of fundamental

[1] *U. S. Egg Poultry Mag.*, 43, 552 (1937).

importance, and sets up a Haugh Unit based on this measurement. This is done by expressing the quality in a per cent system as a negative log of the albumen height, as is shown by line B on Fig. 2. Thus a decrease of 2 mm. in height from 7 to 5 is a quality change of 0.6 scoring units or of 15 per cent in Haugh Units, whereas a decrease of 2 mm. from 3 to 1 is a change of 1.9 scoring units, or of 48 per cent in Haugh Units. However, the area of the thick white shows this same relationship, for the score increases as the logarithm of this area. This is shown in line A, plotted from the data of Parsons and Mink. Calculations of the area from the original pictures of Van Wagenen and Wilgus also give this relationship. Therefore the use of a unit similar to that of Haugh, but based on the area of the thick white, should be satisfactory. The percentage change is best found by a graphical solution, as is done in Fig. 2.

With these considerations in mind the writer attempted to simplify the measurement of the area of thick white. A plate with a very slight uniform concavity was constructed of the following dimensions: 6 inches in diameter with a depth of 1/32 inch to the center. Concentric circles of radii 3–7 cm. were drawn on the blackened surface and protected by a coat of clear shellac. The longest and the shortest axes of the ellipse of thick white can be easily seen and measured by using the concentric circles. The area can then be found in a chart prepared from the equation of an ellipse:

$$A = \pi a \cdot b, \text{ where } a \text{ and } b = 1/2 \text{ the observed axes.}$$

The estimation of the axial distances is not difficult although the outline in many cases deviates from an ellipse. The pictures of Van Wagenen and Wilgus are typical of the various shapes observed. It was found that tilting the plate to make the white more nearly centered, if the yolk is not symmetrically placed, is of advantage and introduces only a negligible error. The axes are read in mm. interpolated from the cm. circles. For convenience the chart of areas for various measurements is set up, the axes as observed being used and not $a/2$ and $b/2$.

The correction for variations in size of the eggs is made in terms of the standard 2-ounce (56.7 grams) egg.

$$\text{Corr. Area} = \text{Observed Area} \cdot \frac{56.7}{\text{Wt. of egg}}.$$

The validity of this correction is discussed subsequently.

EXPERIMENTAL

A test run of this method was made on 350 eggs, the standard photographs of Van Wagenen and Wilgus being used for the comparison. Fresh white Leghorn eggs were obtained from the Bureau of Animal Industry flock at the National Agricultural Research Center, Beltsville, Maryland. The hens were confined to laying houses (January) and fed a well-

balanced scratch-mash diet, containing cod-liver oil. The eggs were collected frequently, chilled to 60° F., and held until the case was completed (3–4 days). These eggs were allowed to depreciate in quality somewhat by standing one day at 80° F. They were than chilled to 50° F. and candled[1] by a grader of the Bureau of Agricultural Economics. All the eggs were broken out and examined by the two methods within 24 hours after candling. The principal difficulty in this comparison is caused

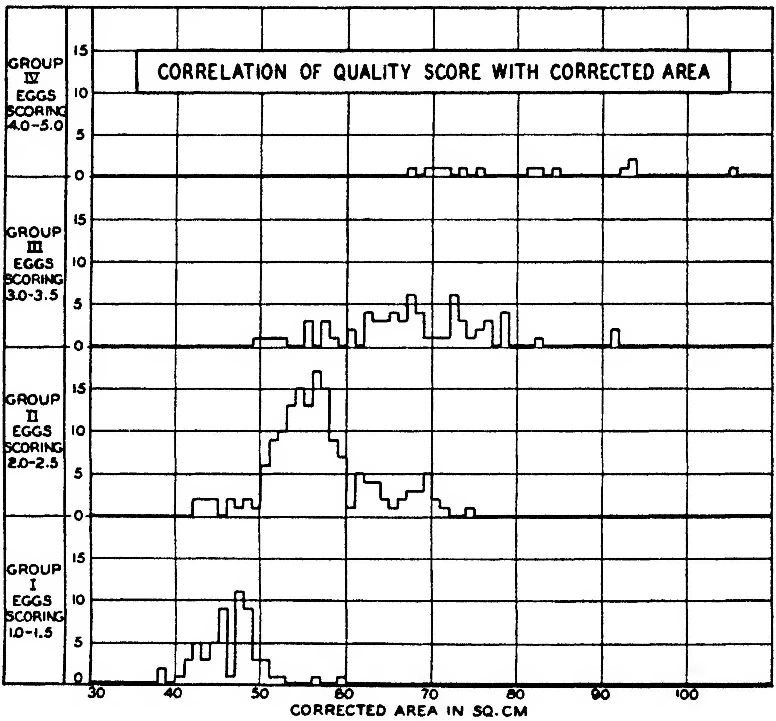

FIG. 3

by the lack of any absolute standard whereby to grade the eggs. An attempt to evolve the objective method proposed here had to be correlated with the subjective one now in use. This renders any exact treatment of the data futile. They are accordingly presented in the form of a distribution diagram (Fig. 3), in which the quality scores are grouped in the manner employed by Parsons and Mink.

The data show a rather sharp differentiation in the eggs of higher quality, and the means of all grades agree very well with those of Parsons and Mink plotted on Fig. 2. In the lower quality grades the correlation is

[1] Due to the nature of the experiment the variations in quality observed by candling were not so great as those commonly encountered in commercial eggs. This was noted by the grader at the time. The weight of the individual egg was neglected in the candling. So few Trades were found that they were discarded.

satisfactory but the spread is relatively greater. A precise evaluation of the whole range would entail measurement of many eggs and should be done by workers more experienced in scoring. Two further points, however, may be obtained from these data.

The largest sample in any group, that of Group 2, was broken down to determine whether the linear correction for weight was satisfactory in this case. The sub-groups of 43–49, 50–59, and 60–63 were evaluated, as shown in Table 1. For eggs of the weights considered, the agreement of the corrected areas indicates that the linear correction as computed from the weight is satisfactory.[1] A larger number of observations over a wider range would be necessary to establish whether an additional correction might be necessary in the case of very small or very large eggs. Haugh found an exponential function of the weight to fit the measurements of Heiman and Carver on the height of the albumen.

Although Van Wagenen and Wilgus showed the scoring technic to correlate with that of candling, the overlapping is very wide. As is shown in Table 2, the measured area of the thick white does not correlate with the candling grade. The agreement of the means and of the medians and the fact that the data for each candling grade group themselves in widely overlapping ranges indicate that the three candling grades are essentially the same when judged by the area standard.[2]

A trial of eggs held in storage for 6 months after various processing treatments indicated that this method has limitations in such an application. These eggs had an atypical appearance judged by the photographic score; the thick white area was small and the per cent thick was very definitely lower than it is in fresh eggs. *In outline* these eggs appeared to be much better than they did *in general appearance*, because the thick white decreases in storage, although that part remaining around the yolk holds up fairly well. The effect is to give the appearance of a small and fairly firm area of thick white, which is characteristic of the thick white of a fresh egg. The storage egg, therefore, is rated higher on the basis of the area of thick white observed, although there is a lessened amount due to the deterioration of the egg. Incidentally, the same objection applies in some measure to the use of the photographic score and to any method that takes account of the area of thick white.

DISCUSSION

The measurement of the area of the thick white used as an index of interior quality of eggs has the following advantages: It is a simple visual observation that can be made quickly and calculated from charts that are easily prepared. For rough work by those experienced in handling eggs the weight can be approximated. The area that is measured seems

[1] Agreement exists between mean and median corrected areas, and the range of the data (i.e., the spread between the smallest and the largest observation) is the same in each group

[2] It should be noted that the recognition of imperfect eggs, which is one of the principal objects of candling, is not here taken into account.

to be the controlling factor in the visual judgment of quality. Results can be expressed in a per cent quality system, which is often advantageous. The measurement is objective and therefore lends itself to standardization investigations.

TABLE 1.—*Agreement in area measurements after correction for the weight of the egg*

WEIGHT GROUP	NUMBER IN GROUP	MEAN AREA CORRECTED FOR WEIGHT	MEDIAN AREA CORRECTED FOR WEIGHT	MAXIMUM AND MINIMUM AREAS CORRECTED WITHIN THE GROUP
grams		*sq. cm.*	*sq. cm.*	*sq. cm.*
43–49	17	55.7	55.0	43–74
50–59	116	57.0	55.5	42–71
60–63	27	53.8	56.0	42–63

TABLE 2.—*Lack of correlation between candling grades and area measurements*

CANDLING GRADE	NUMBER IN GROUP	MEAN CORRECTED AREA	MEDIAN CORRECTED AREA	RANGE OF CORRECTED AREAS
Special	44	57.6	56	42–84
Extra	116	56.5	55	38–125
Standard	93	59.8	57	41– 92

SUMMARY

A logarithmic relationship between the area of thick white and the "broken-out quality" of eggs has been observed. The significance of this area has been pointed out and the method of its determination simplified.

INTERPRETATION OF CHEMICAL ANALYSES OF PRESERVES AND JAMS*

By J. W. SALE† (U. S. Food and Drug Administration, Washington, D. C.)

To determine the fruit content of market samples of alleged preserves,[1] accurate and appropriate chemical analyses of the samples and a comprehensive knowledge of the chemical composition of fruits of the kinds declared on the labels are necessary.

DIAGNOSTIC CONSTITUENTS OF PRESERVES

Methods for the chemical analysis of preserves are contained in *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, 4th edition (1935) and in *This Journal*. Regulation 4

* Contribution from Food Division.
† In Charge, Beverage Section, Food Division.
[1] The term "preserve" as used in this paper includes "jam." A product in which the fruit is whole or in relatively large pieces is customarily designated a "preserve" rather than a "jam."

of the Federal Food and Drugs Act states that all foods shall be analyzed by the methods prescribed by the Association of Official Agricultural Chemists, when applicable, provided, however, that any method of analysis or examination satisfactory to the Food and Drug Administration may be employed.

Water-insoluble solids, total ash or ash of the water-soluble portion, potash, and phosphate are criteria of the fruit content, as will be explained later. Whether total ash or ash of the water-soluble portion is to be determined will depend upon which of these constituents was determined on the samples of fruits used for comparison. Per cent total ash in the preserves should be compared with per cent total ash in the fruit, and per cent ash of the water-soluble portion of the preserve should be compared with the ash of the water-soluble portion of the corresponding fruit.[1] Total reducing sugars after inversion should be determined in order that the degree of concentration may be calculated, as will be explained later. The determination of soluble solids is useful for this purpose also. If the non-sugar solids are desired, total solids and sucrose should be determined also, since non-sugar solids are the total solids less the sum of the sucrose and invert sugar in the sample. Non-sugar solids have not been used here as an index of fruit content of preserves for many years, because constituents that can be determined directly and with greater accuracy have been shown to reflect the fruit content more closely. Determination of alcohol precipitate, pectic acid, and total acidity will show whether or not added pectinous substance and added acid are present. When glucose, artificial color, or fruit other than that named on the label are suspected, glucose, artificial color, and the characteristic acid or acids of the named fruit should be determined. Microscopic examination will usually show the presence of foreign fruit tissue. The report of analysis should include a statement of the odor, taste, and appearance of the sample. Occasion will arise when other constituents, such as sulfur in ash, lactic acid, etc., should be determined, but the analyses mentioned will suffice to illustrate the method of arriving at the proportion of fruit used in the manufacture of a preserve, the subject of chief concern here.

COMMENTS ON METHODS OF CHEMICAL ANALYSIS OF PRESERVES

The laboratories of the Food and Drug Administration in Washington and in the field analyze a large number of market samples of preserves and authentic fruits each year, and in the course of this work questions arise as to details of procedure that are not fully covered in the methods of the A. O. A. C. The following description of many of these details of laboratory technic followed in the Administration may prove helpful to chemists who infrequently have occasion to analyze preserves.

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[1] The unqualified term "ash" as used in this paper means the ash of the water-soluble portion of the sample.

Preparation of sample for analysis.—The sample of preserve is prepared for analysis by the procedure described in *Methods of Analysis, A.O.A.C.*,[1] p. 319, **2** (c) and (c_1). The burrs or plates of the food chopper are closely set, but in grinding preserves made from berry fruits, the cracking of the seeds is avoided as much as possible. The sample is passed through the food chopper three times. If the container is a No. 10 can or smaller, the entire contents are ground; if the containers are larger than No. 10 cans, the contents are stirred thoroughly and a portion is removed for grinding.

In the case of preserves containing pits or large seeds such as Damson plum preserves, grape jam, etc., the sample is pulped as thoroughly as possible in a mortar, without crushing the pits or seeds. Large portions of approximately 300 grams each are weighed. One portion is used to prepare the sample solution. The other portions are used for the determination of insoluble solids.

Insoluble solids.—The insoluble-solids are determined as directed in *Methods of Analysis, A.O.A.C.*, p. 320, **7**. At least two determinations of insoluble solids are made on each sample; 300 gram portions of samples of Damson plum preserves, grape jam, and other preserves that contain pits or large seeds, prepared for analysis as described above, are used for the determination instead of the 25 grams prescribed by the A.O.A.C. method. The cotton pad and washed pulp together with the pits, or large seeds, are dried and weighed. The pits or seeds are then picked out, soaked thoroughly in water, freed from any adhering pulp, dried at 100° C., and their weight and the weight of the cotton pad are subtracted from the gross weight of pad and residue, to obtain the weight of insoluble solids in the 300 gram portion of the sample. The filtering medium provided for in the method is absorbent cotton or filter paper. A weighed piece of cotton 5 inches square and of a thickness about one-half that of the layer in the ordinary 16 ounce roll of absorbent cotton is used for the 25 gram samples. Such a piece of cotton weighs about 4 grams. A piece of the cotton is torn off one corner and is used to plug the neck of the funnel lightly. The large piece is then arranged in the funnel, and the sample is filtered, care being taken that all the pulp remains on the cotton. The hot distilled water is poured on in such a way that the pulp is loosened from the cotton with each addition. Usually 700–800 cc. of filtrate is collected. The cotton is folded carefully to enclose all particles of pulp and excess water is removed by gently squeezing it while it is still in the funnel. The cotton plug from the neck of the funnel and the remainder of the cotton and adhering pulp are dried to constant weight at 100° C.

Soluble solids.—The procedure for soluble solids in *Methods of Analysis, A.O.A.C.*, p. 320, **8**, is followed. A ground-up sample of preserve and a refractometer of the Abbe' type are used. Reports of collaborative work of this Association, *This Journal*, **15**, 375–384 (1932); **17**, 207 (1934), show that the refractometric method is at least as accurate as methods based on evaporation of an aliquot of the sample solution and drying of the residue in vacuo at 70° C. The refractometric method has the further advantage of requiring less time than the drying methods.

Ash.—The ash of the water-soluble portion, *i.e.*, sample solution, is determined as directed in *Methods of Analysis, A.O.A.C.*, p. 321, **9**, and p. 336, **8**, with the temperature of ashing not over 525° C. When excessive swelling or foaming is encountered, 2 or 3 drops of ashless olive oil are added as provided in *Methods of Analysis*, 465, **8** and **9**.. The partially ashed residues are wetted to shorten time of ashing. Without refiltering, the wetted residue is dried on the steam bath and hot plate and reheated in the muffle. Samples of preserves containing added H_3PO_4 are never ashed in platinum dishes unless excess alkali is added as in *Methods of Analysis, A.O.A.C.* p. 326, **12**. The presence of added H_3PO_4 is shown by an ash with acid reaction, which remains black and cinder-like after repeated wettings.

[1] In all references to *Methods of Analysis, A.O.A.C.* in this paper the 1935 edition is meant.

Potash (K_2O).—Potash is determined in the ash as directed in *Methods of Analysis, A.O.A.C.*, p. 321, **14** and **15**, which is a modification of the Lindo-Gladding method. This method gives accurate results with large or small quantities of potassium, *This Journal*, **11**, 447 (1928); **12**, 368 (1929); **14**, 463 (1931).

Phosphate (P_2O_5).—Phosphate is determined in the ash as directed in *Methods of Analysis, A.O.A.C.*, p. 166, **19**, and p. 21, **12**. Both Gooch crucibles and filter papers are used for filtering the precipitate. Blanks are run on the asbestos and filtering paper. When free P_2O_5 is present, excess alkali is added before ashing as described above under "Ash" to avoid loss of P_2O_5 or potassium salts or harm to platinum dishes, *This Journal*, **8**, 630 (1925).

Alcohol precipitate and pectic acid.—The procedures in *Methods of Analysis, A.O.A.C.*, p. 324, **21** and **22**, are followed. The second precipitation of alcohol precipitate is allowed to stand 1 hour or longer. The alcohol precipitate is not permitted to dry before it is transferred from the filter papers. When the preserve contains commercial glucose the alcohol precipitate will be copious, gummy, and sticky, due to dextrins.

Total acidity.— The procedure in *Methods of Analysis, A.O.A.C.*, p. 325, **24**, is followed, except that a larger aliquot of the sample solution is usually taken for titration to obtain greater accuracy.

Total sugars after inversion.—An aliquot of the sample solution is clarified with neutral Pb acetate, the Pb removed from the filtrate, and the sucrose in the lead-free filtrate inverted by the procedures described in *Methods of Analysis, A.O.A.C.*, p. 469, **18** (d); p. 476, **28**; and p. 473, **23** (b) or (c), respectively. In filtering through folded filters after addition of the neutral Pb acetate, and also after addition of dry sodium or potassium oxalate, the first portion of the filtrate is discarded. Watch-glasses are placed on the funnels and beakers during these filtrations to avoid evaporation. The total sugars after inversion are then determined gravimetrically by the Munson and Walker method, *Methods of Analysis, A.O A.C.*, p. 479, **37**, or volumetrically by the Lane-Eynon method in the same book p. 478, **33**. If a large number of samples of preserves are to be examined, the Lane-Eynon method is preferred. The two methods give equally accurate results.

CHEMICAL COMPOSITION OF FRUITS

Realizing many years ago the need of authentic data on the chemical composition of fruits, the U. S. Food and Drug Administration and its predecessor, the Bureau of Chemistry, began to collect and analyze representative samples of fruits of the kind used in preserves and jellies. The work was begun on a broad scale in 1919 and has been continued more or less regularly throughout the intervening years. Approximately 1000 such samples have been collected and analyzed under this project. Each of the samples is known to be free from added sugar, excess water, or other foreign substance. The resulting data, with the exception of those on fruits which are seldom, if ever, used in preserves (blueberries, cranberries, citrus fruits, pears, and pomegranates), covering 774 samples, are summarized in Tables 1 and 2, which are self-explanatory.

Minima and maxima results have not been included in the tables, since they are not used in determining the fruit content of preserves by the procedure described herein. File copies of the voluminous individual analyses may be consulted at the U. S. Food and Drug Administration, Washington, D. C.

DETERMINATION OF FRUIT CONTENT OF PRESERVES FROM THEIR CHEMICAL ANALYSES

When a quantity of fruit and a quantity of granulated sugar are concentrated by cooking, the resulting mixture will contain the same quantities of water-insoluble solids (seeds and fibrous material), ash (mineral matter), potash, and phosphate that were present in the amount of fruit taken, since granulated sugar will not contribute to the mixture appreci-

TABLE 1.—*Averages of results on fruits of known origin (i.e., authentic fruits) collected and analysed by U. S. Food and Drug Administration**

KIND OF FRUIT	TOTAL SUGARS AS INVERT	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	ASH OF SAMPLE SOLUTION	PHOSPHATE (P_2O_5)	
					mg./100 grams	*per cent in ash*
	per cent	*per cent*	*per cent*	*per cent*		
Apples	11.1 (29)†	13.7 (38)	2.34 (25)	0.32 (38)	24 (37)	7 (37)
Apricots	9.8 (40)	14.4 (40)	1.66 (40)	.80 (42)	56 (41)	7 (41)
Blackberries	7.0 (54)	10.0 (54)	6.24 (54)	.46 (55)	43 (53)	9 (53)
Cherries	9.3 (38)	13.9 (37)	1.41 (45)	.48 (52)	44 (45)	9 (45)
Crabapples	11.2 (30)	15.4 (30)	2.98 (18)	.41 (30)	36 (30)	9 (30)
Currants	6.0 (35)	10.6 (35)	5.71 (25)	.58 (35)	48 (31)	8 (31)
Figs	15.8 (16)	19.0 (16)	2.76 (16)	.55 (16)	47 (14)	8 (14)
Gooseberries	3.9 (8)	8.2 (8)	3.13 (8)	.43 (8)	29 (8)	7 (8)
Guavas	4.3 (24)	7.6 (24)	10.24 (24)	.62 (24)	39 (24)	6 (24)
Grapes	11.6 (31)	14.1 (31)	1.47 (19)	.52 (32)	30 (31)	6 (31)
Loganberries	6.3 (35)	10.6 (35)	5.75 (35)	.50 (35)	44 (35)	9 (35)
Peaches	8.8 (33)	11.8 (33)	1.40 (33)	.49 (34)	44 (34)	9 (34)
Pineapples	12.8 (40)	14.6 (40)	1.12 (40)	.43 (40)	14 (40)	3 (40)
Plums	7.4 (40)	14.8 (40)	1.52 (40)	.57 (40)	38 (40)	7 (40)
Quinces	7.6 (19)	13.2 (21)	4.58 (11)	.44 (21)	37 (21)	8 (21)
Raspberries (Red)	7.2 (58)	10.5 (57)	5.89 (57)	.45 (60)	47 (59)	11 (59)
Raspberries (Black)	7.1 (15)	11.2 (15)	8.85 (15)	.56 (17)	53 (17)	10 (17)
Strawberries	5.3 (172)	8.0 (164)	2.69 (196)	.46 (195)	44 (182)	9 (182)

KIND OF FRUIT	POTASH (K_2O)		ALCOHOL PRECIPITATE	PECTIC ACID	TOTAL ACIDITY		
					0.1 N/100 grams	CALCULATED	
	mg./100 grams	*per cent in ash*	*per cent*	*per cent*	*cc.*	*per cent*	*as—*
Apples	166 (12)	45 (12)	0.71 (38)	0.38 (38)	77 (38)	0.52 (38)	malic
Apricots	454 (20)	54 (20)	.81 (40)	.46 (37)	168 (40)	1.13 (40)	malic
Blackberries	235 (15)	49 (15)	.58 (55)	.32 (55)	170 (52)	1.09 (52)	isocitric
Cherries	248 (20)	51 (20)	.20 (47)	.09 (47)	203 (46)	1.36 (46)	malic
Crabapples			1.09 (30)	.59 (30)	159 (30)	1.07 (30)	malic
Currants			.61 (35)	.39 (34)	334 (34)	2.14 (34)	citric
Figs			.89 (16)	.49 (16)	31 (15)	.20 (15)	citric
Gooseberries			.82 (8)	.52 (8)	371 (8)	2.37 (8)	citric
Guavas			.90 (24)	.50 (24)	136 (24)	.87 (24)	citric
Grapes			.41 (32)	.26 (32)	159 (31)	1.19 (31)	tartaric
Loganberries			.69 (35)	.37 (35)	342 (35)	2.19 (35)	citric
Peaches	246 (14)	51 (14)	.82 (32)	.40 (32)	93 (33)	.62 (33)	malic
Pineapples	197 (21)	45 (21)	.12 (40)	.04 (40)	122 (39)	.78 (39)	citric
Plums			1.16 (40)	.70 (40)	330 (40)	2.21 (40)	malic
Quinces			1.03 (21)	.61 (21)	146 (21)	.98 (21)	malic
Raspberries (Red)	193 (29)	44 (29)	.63 (58)	.30 (58)	210 (58)	1.35 (58)	citric
Raspberries (Black)			.65 (17)	.35 (17)	162 (17)	1.04 (17)	citric
Strawberries	209 (25)	50 (25)	.53 (193)	.33 (193)	174 (197)	1.11 (197)	citric

* Predecessor—Bureau of Chemistry.
† Numbers in parentheses denote the number of samples analysed.

able amounts of any of the constituents in question and none of these constituents will be lost in the process of cooking. Analyses of experimental batches of preserves made in the Food and Drug Administra-

TABLE 2.—*Source and time of collection of authentic fruits in Table 1*
(Figures in parentheses denote the number of samples)

Apples: Calif. (4); Colo. (12); Mich. (1); N. Y. (2); Oregon (5); Wash. (13); and Utah (1). 1922 (3); 1923 (32); year not reported (3).
Apricots: Calif. (40); Utah (1); Wash (1); 1924 (17); 1925 (6); 1935 (19).
Blackberries: Calif. (17); Colo. (4); Fla. (2); Ga. (4); La. (3), 1919 (1); 1921 (3); 1922 (19); 1923 (1); 1924 (2); 1926 (1); 1927 (2); 1935 (21); year not reported (5).
Cherries: Colo. (4); Ill. (1); Mass. (1); Mich. (7); Mo. (2); N. J. (3); N. Y. (14); Ohio (5); Oregon (1); Pa. (1); Wash. (7); Wis. (3); state not reported (3). 1919 (2); 1924 (15); 1925 (22); 1935 (9); 1937 (4).
Crabapples: Calif. (4); Ill. (1); Mich. (14); Wash. (11). 1923 (1); 1925 (1); 1935 (27); year not reported (1).
Currants: Calif. (1); Colo. (5); Md. (1); Mass. (2); Mich. (2); N. J. (1); N. Y. (10); Ohio (2); Oregon (1); Pa. (1); Wash. (6). State not reported (3); 1923 (10); 1924 (9); 1925 (4); 1935 (8); year not reported (4).
Figs.: Calif. (10); La. (4); Tex. (2). 1923 (1); 1924 (4); 1925 (5); 1926 (3); 1935 (3).
Gooseberries: Colo. (1); N. J. (2); Wash. (5). 1923 (4); 1924 (2); 1935 (2).
Guavas: Fla. (24). 1935 (2); 1936 (18); 1937 (4).
Grapes: Ark. (3); Calif. (2); Colo. (1); Mich. (5); Mo. (7); Wash. (4). State not reported (10); 1919 (1); 1923 (2); 1924 (2); 1927 (6); 1935 (19); year not reported (2).
Loganberries: Calif. (7); Oregon (9); Wash. (19). 1921 (5); 1922 (11); 1935 (19).
Peaches: Ala. (1); Calif. (18); Colo. (2); Ga. (4); Mich. (5); N. Y. (1); Ohio (1); Tex. (1); Wash. (1); 1924 (10); 1925 (9); 1935 (11); year not reported (4).
Pineapples: Hawaii (37); P. R. (3). 1922 (2); 1924 (2); 1925 (3); 1935 (33).
Plums: Calif. (11); Colo. (1); Ill. (1); Mich. (11); Mo. (1); N. Y. (1); Ohio (1); Wash. (1); State not reported (12). 1923 (2); 1924 (3); 1925 (1); 1935 (31); year not reported (3).
Quinces: Calif. (6); Md. (2); Mich. (8); N. Y. (3); Oregon (2). 1923 (1); 1926 (5); 1935 (15).
Raspberries (Red); Calif. (9); Colo. (6); Mich. (5); N. J. (5); N. Y. (8); Oregon (1); Wash. (25). State not reported (1); 1919 (1); 1922 (5); 1923 (7); 1925 (17); 1935 (22); 1937 (6); year not reported (2).
Raspberries (Black): Colo. (1); Mich. (2); N. Y. (4); Oregon (3); Wash. (7); 1923 (2); 1935 (13). Year not reported (2).
Strawberries: Calif. (18); Colo. (12); Del. (3); Fla. (5); Ind. (1); Ky. (2); La. (8); Md. (12); Mich. (3); Minn. (1); Mo. (1); N. C. (4); N. J. (21); N. Y. (17); Oregon (17); Tenn. (12); Utah (3); Va. (17); Wash. (16); Wis. (1); state not reported (24); 1921 (23); 1922 (15); 1923 (53); 1924 (16); 1925 (17); 1926 (4); 1927 (8); 1930 (1); 1935 (58); 1938 (3).

tion and the Bureau of Chemistry have shown that the percentages of these constituents in the mixture are in strict proportion to the amount of fruit used. Furthermore, in making up experimental batches in preserve factories and in the laboratory it has been found that the content of fruit in concentrated mixtures of fruit and sugar will be closely approximated

TABLE 3.—*Results on supervised factory packs of standard and sub-standard preserves**

SAMPLE	KIND OF FRUIT	TOTAL SUGARS AS INVERT	INSOLUBLE SOLIDS	ASH OF SAMPLE SOLUTION	PHOSPHATE (P_2O_5)		POTASH K_2O	
		per cent	*per cent*	*per cent*	*mg./100 grams*	*per cent in ash*	*mg./100 grams*	*per cent in ash*
8161-D	Apricot	69.2	0.97	0.41	28.2	6.9	225.9	55.1
8162-D	Apricot	70.7	0.96	0.38	27.9	7.3	215.2	56.6
8163-D	Peach	68.0	0.23	0.17	10.7	6.5	60.0	36.4
8164-D	Peach	75.9	0.30	0.14	10.0	7.1	60.1	42.8
8165-D	Pineapple	68.1	0.49	0.20	6.8	3.4	69.0	34.5
8166-D	Pineapple	70.8	0.49	0.17	7.0	4.1	60.7	35.7
8167-D	Raspberry	70.8	2.92	0.26	17.1	6.6	118.3	45.5
8168-D	Raspberry	67.8	3.15	0.24	19.2	8.0	118.8	49.5
8169-D	Strawberry	70.1	1.02	0.22	14.0	6.4	92.5	42.0
8170-D	Strawberry	71.5	0.99	0.18	13.2	7.3	83.1	46.2
8171-D	Blackberry	69.0	2.32	0.26	12.9	5.0	103.1	39.7
8172-D	Blackberry	71.3	2.46	0.23	13.3	5.8	99.4	43.2

* Odd-numbered samples were made from partially refined commercial sugar sirups Even-numbered samples were made from granulated sugar. Apricots and peaches were from No. 10 tins labeled "Solid pack." Pineapples were from No. 10 tins labeled "Crushed pineapple in juice" Raspberries, strawberries, and blackberries were in crates out of cold storage All samples except 8161-2-7-8-D contained added citric acid and citrus pectin (100 grade).

when the average values of two or more of the diagnostic constituents mentioned above are used as a basis of comparison (Table 1).

The total acidity and pectic acid of a market sample are not dependable indices of the fruit content when the ratios of certain constituents to each other show that the sample contains added acid and added pectin, as explained below.

For the customary pure granulated sugar, some manufacturers of preserves and jams substitute a partially refined sugar sirup, sometimes called "liquid sugar." This product contains varying amounts of mineral matter (ash), depending upon the degree of refinement. When it is used in the preparation of an alleged preserve, the ash in the finished product will be obtained from both the fruit and the "liquid sugar." It is also a common practice in the preserving industry for manufacturers to add commercial pectin in the manufacture of alleged preserves to thicken them, and this substance will contribute some mineral matter to them. Other variations from the normal practice of manufacture of preserves that must be considered are the use of glucose, acid phosphate, and artificial color. Evidence of the presence of these substances will be found in the ratio of various constituents in the alleged preserve to each other. Added pectin and added acid will be indicated when the ratios of pectic acid to ash $\left(\frac{\%\ \text{pectic acid}}{\%\ \text{ash}}\right)$ and of total acid to ash $\left(\frac{\%\ \text{total acid}}{\%\ \text{ash}}\right)$ are abnormal-

ly high (*i.e.*, well above the average). When these ratios are abnormally low and the ratio of ash to potash $\left(\frac{\% \text{ ash}}{\% \ K_2O}\right)$ is in each case abnormally high, the presence of substances other than fruit is indicated. Abnormally high ratios of alcohol precipitate to ash $\left(\frac{\% \text{ alcohol precipitate}}{\% \text{ ash}}\right)$ and of alcohol precipitate to pectic acid $\left(\frac{\% \text{ alcohol precipitate}}{\% \text{ pectic acid}}\right)$ indicate the presence of glucose, but it should be confirmed by the determination of commercial glucose. Abnormally high ratios of insoluble solids to ash or to potash $\left(\frac{\% \text{ insoluble solids}}{\% \text{ ash or } \% \ K_2O}\right)$ indicate either the use of fruit from which the juice has been drained off, or the addition of fruit pomace.

The effect of using "liquid sugar" and commercial pectin is clearly shown in Tables 3 and 8.

In order to be certain that there is actual and substantial fruit shortage in a shipment of alleged preserves the Food and Drug Administration takes into account the various available measures of fruit content and also examines several different batches from the same manufacturer. In this connection it should be borne in mind that the ratio of 45 pounds of fruit to each 55 pounds of sugar in the standard *is a minimum ratio* with respect to the fruit content.

Typical supporting data for the conclusions stated previously are contained in Tables 3–8, inclusive. These tables are self-explanatory except in regard to part of the data in Table 8, which is explained under the caption "Pounds of fruit used to each 55 pounds of sugar in the original batch." The batches of preserves considered in Tables 3–8, inclusive, were prepared in a factory in Brooklyn, N. Y., in February 1938, and analyzed by chemists of the Food and Drug Administration. They represent a number of different fruits and manufacturing formulas. These results are similar to those obtained on other experimental batches, and are in accord with the experience of the Administration extended over a period of many years.

The fallacy of using the absolute minima of the various diagnostic constituents in arriving at the fruit content of an alleged preserve can be illustrated by applying this procedure to the analyses of the supervised factory packs of substandard and standard preserves (Table 3). For example, the first sample in this table, No. 1861–D, is a substandard apricot pre-

TABLE 4.—*Ash, phosphate, and potash contributed by sugar sirup and pectin to the standard and substandard preserves in Table 3**

	USED IN PRESERVE NO.		ASH	PHOSPHATE (P_2O_5)	POTASH (K_2O)
			per cent	*mg./100 grams*	*mg./100 grams*
Sirup No. 8179-D	8161-D	Apricot	0.037		
Pectin	8161-D	Apricot	None used		
Sirup	8162-D	Apricot	None used		
Pectin	8162-D	Apricot	None used		
Sirup No. 8179-D	8163-D	Peach	0.037		12
Pectin Solution No. 8180-D	8163-D	Peach	0.031	0.7	1
Sirup	8164-D	Peach	None used		
Pectin Solution No. 8180-D	8164-D	Peach	0.035	0.7	1
Sirup No. 12006-D	8165-D	Pineapple	0.038	0.3	10
Pectin, Dry, No. 12003-D	8165-D	Pineapple	0.028	0.3	3
Sirup	8166-D	Pineapple	None used		
Pectin, Dry No. 12003-D	8166-D	Pineapple	0.029	0.3	3
Sirup No. 8179-D	8167-D	Raspberry	0.038		12
Pectin	8167-D	Raspberry	None used		
Sirup	8168-D	Raspberry	None used		
Pectin	8168-D	Raspberry	None used		
Sirup No. 8179-D	8169-D	Strawberry	0.039		13
Pectin Solution No. 8180-D	8169-D	Strawberry	0.033	0.7	1
Sirup	8170-D	Strawberry	None used		
Pectin Solution No. 8180-D	8170-D	Strawberry	0.033	0.7	1
Sirup No. 12006-D	8171-D	Blackberry	0.041	0.4	11
Pectin, Dry No. 12003-D	8171-D	Blackberry	0.018	0.2	2
Sirup	8172-D	Blackberry	None used		
Pectin, Dry No. 12003-D	8172-D	Blackberry	0.019	0.2	2

* Ash contributed by added citric acid was less than 0.001% in all cases. These corrections are based on analyses of the partially refined sugar sirups and pectin samples and the amounts of them used in making the standard and substandard preserves.

TABLE 5.—*Results on supervised factory packs of standard and substandard preserves corrected where necessary for added ash, phosphate, and potash derived from partially refined sugar sirup and pectin**

PRESERVE NO.	KIND OF FRUIT	TOTAL SUGARS AFTER INVERSION	INSOLUBLE SOLIDS	ASH OF SAMPLE SOLUTION	PHOSPHATE (P_2O_5)	POTASH (K_2O)
		per cent	*per cent*	*per cent*	*mg./100 grams*	*mg./100 grams*
8161-D	Apricot	69.2	0.97	0.37	28	214
8162-D	Apricot	70.7	0.96	0.38	28	215
8163-D	Peach	68.0	0.23	0 10	10	47
8164-D	Peach	75.9	0.30	0.10	9	59
8165-D	Pineapple	68.1	0.49	0.13	6	56
8166-D	Pineapple	70.8	0.49	0.14	7	58
8167-D	Raspberries	70.8	2.92	0.22	17	106
8168-D	Raspberries	67.8	3.15	0.24	19	119
8169-D	Strawberries	70.1	1.02	0.15	13	78
8170-D	Strawberries	71.5	0.99	0.15	12	82
8171-D	Blackberries	69.0	2.32	0.20	12	90
8172-D	Blackberries	71.3	2.46	0.21	13	97

* Based on data in Tables 3 and 4.

TABLE 6.—*Results on fruits used in making factory packs of standard and substandard preserves*

SAMPLE NO.	KIND OF FRUIT	TOTAL SUGARS AFTER INVERSION AS INVERT SUGAR	INSOLUBLE SOLIDS	ASH OF SAMPLE SOLUTION	PHOSPHATE (P_2O_5)		POTASH (K_2O)	
		per cent	*per cent*	*per cent*	*mg./100 grams*	*per cent in ash*	*mg./100 grams*	*per cent in ash*
8173-D	Apricot*	8.20	2.07	0.86	60.2	7.0	465.5	54.1
8174-D	Peach*	10.85	1.09	0.50	46.0	9.2	242.9	48.6
8175-D	Pineapple*	14.26	1.26	0.35	15.2	4.3	149.8	42.8
8176-D	Raspberry†	5.93	5.81	0.49	39.5	8.1	225.3	46.0
8177-D	Strawberry†	4.98	3.37	0.53	44.0	8.2	259.6	48.6
8178-D	Blackberry†	5.43	6.61	0.55	33.8	6.1	266.9	48.5

* Canned.
† Fresh, frozen.

serve made from 40.6 pounds of fruit to each 55 pounds of sugar. If the minimum insoluble solids of the 42 authentic samples of apricots, which the records of this Administration show to be 1.23 per cent, is used for comparison, there would be obtained an apparent fruit content of 74 pounds of fruit to each 55 pounds of sugar, nearly double the amount of

fruit actually used. Such minima (exceptional by definition) are not a factor in interpreting the analyses of preserves made from batches of commercial size.

TABLE 7.—*Amounts of fruit and sugar used in making factory packs of standard and substandard preserves, and yields*

PRESERVE NO.	KIND OF FRUIT	FRUIT USED	SAMPLE NO. OF FRUIT USED	SUGAR USED	SUGAR SIRUP USED	TOTAL SUGARS AS INVERT IN SUGAR SIRUP USED	YIELD
		pounds		*pounds*	*pounds*	*per cent*	*pounds*
8161-D	Apricot	16	8173-D		30	76.1	33.2
8162-D	Apricot	16	8173-D	22			33.4
8163-D	Peach	12	8174-D		45.2	76.1	49 9
8164-D	Peach	12	8174-D	33			45.5
8165-D	Pineapple	7	8175-D		15.	75.8	18.1
8166-D	Pineapple	7	8175-D	11			17.9
8167-D	Raspberries	18	8176-D		34.	76.1	36.9
8168-D	Raspberries	18	8176-D	22			34.6
8169-D	Strawberries	15	8177-D		46.	76.1	48.2
8170-D	Strawberries	15	8177-D	33			47.8
8171-D	Blackberries	6	8178-D		15.	75.8	16.9
8172-D	Blackberries	6	8178-D	11			16.6

TABLE 8.—*Pounds of fruit to 55 pounds of sugar in factory packs of standard and substandard preserves*

PRESERVE NO.	BATCH NO.	KIND OF FRUIT	LBS. FRUIT TO 55 LBS. OF SUGAR— BASED ON AVERAGE ANALYSES OF AUTHENTIC FRUITS AND ON ANALYSES OF THE SAMPLES (1)	BASED ON ANALYSES OF FRUITS ACTUALLY USED AND ON CORRECTED ANALYSES OF THE SAMPLES (1)	BASED ON ACTUAL WEIGHTS OF FRUIT AND SUGAR USED (2)
8161-D	1	Apricot	47 (3)	42	40.6
8162-D	2	Apricot	45	40	40.
8163-D	3	Peach	22 (3)	18	20.2
8164-D	4	Peach	19	18	20
8165-D	5	Pineapple	41 (3)	35	35.6
8166-D	6	Pineapple	36	37	35.
8167-D	7	Raspberry	44 (3)	39	40.3
8168-D	8	Raspberry	47	45	45.
8169-D	9	Strawberry	34 (3)	25	24.8
8170-D	10	Strawberry	30	24	25.
8171-D	11	Blackberry	37 (3)	30	30.6
8172-D	12	Blackberry	34	31	30.

(1) Using insoluble solids, ash, P_2O_5, and K_2O for comparison. See Tables 1, 3, 5, and 6 for data used in these calculations.
(2) Calculated from data in Table 7.
(3) Made from partially refined commercial sugar sirups, which contributed ash material to the samples. All the samples except 8161-2-7-8-D contained added pectin, which contributed ash material also. The added ash material from these sources increased the apparent fruit content, based on the uncorrected analysis.

POUNDS OF FRUIT USED TO EACH 55 POUNDS OF SUGAR IN THE ORIGINAL BATCH

Since it is customary in the industry to express the composition of a preserve in terms of pounds of fruit used to each 55 pounds of sugar in the original batch, it is convenient for ready comparison to express the proportion of fruit used in the manufacture of a sample of alleged preserves in the same manner. A detailed example of the method of arriving at the amounts of fruit and sugar used in the manufacture of an alleged preserve, using as a basis its ash content and the average ash content of the corresponding fruit, follows:

100 lbs. of alleged preserve contains 0.2 lb. ash (actual analysis).

100 lbs. of the fruit contains 0.5 lb. ash (average of authentics).

If 100 lbs. of the fruit contains 0.5 lb. of ash, how much of that fruit will contain 0.2 lb. of ash? Expressed in the form of a proportion—$100:0.5::X:0.2$. $X=40$ lbs. fruit.

100 lbs. of alleged preserve contains 70.0 lbs. total invert sugar (actual analysis).

This same alleged preserve contains both added sugar and sugar normal to the fruit used.

100 lbs. of the fruit contains 8.0 lbs. of invert sugar and 1 lb. of it contains 0 08 lb. invert sugar (average of authentics).

Then 40 lbs. of fruit contains 40×0.08 lb. invert sugar or 3.2 lbs. invert sugar in 100 lbs. of alleged preserves, and

70.0 lbs. $-$3.2 lbs. $=$66.8 lbs. added invert sugar in 100 lbs. of alleged preserve.

Since the sugar added as such was in the form of sucrose (ordinary sugar) when the batch was made up, the 66.8 lbs. of invert sugar will be the equivalent of 66.8×0.95, or 63.5 lbs. of sucrose.

40 lbs. of raw fruit concentrated by cooking with 63.5 lbs. of sugar to 100 lbs. will give a product with the composition of the alleged preserve.

If 40 lbs. of raw fruit is used with 63.5 lbs. of sugar, how much raw fruit will be used to 55 lbs. of sugar? Expressed in the form of a proportion—$40:63.5::X:55$. $X=34.6$ lbs. of fruit used to 55 lbs. of sugar in original batch.

In a similar manner, the amounts of fruit and sugar used can be calculated from the insoluble solids, potash, or any other reliable indices of fruit content. The average of the apparent fruit contents calculated from several of these reliable indices is used by the Food and Drug Administration in its regulatory work. This average figure will closely approximate the number of pounds of fruit to each 55 pounds of sugar actually used by the manufacturer, when appropriate consideration is given to the possibility of ash material being present from non-fruit sources, as will be shown by the ratios of the constituents to each other.

ADDED WATER

Preserves that are made from fruit and sugar in the normal proportions and that are concentrated by cooking in the usual way will not contain in their finished form any added water. Moreover, the weight of the fruit and sugar mixture will be reduced 10 per cent or more by the removal of fruit moisture in the process of cooking. The proportion of added water

in an alleged preserve can be readily calculated. The number of pounds of fruit used to each 55 pounds of sugar in the preparation of the alleged preserve, having been determined by the procedure described previously, is multiplied by the average percentage of sugar as invert in the corresponding kind of fruit. The result obtained, pounds of fruit sugar, is added to the pounds of added sugar expressed as "invert sugar," namely 57.9, and the sum is the pounds of total sugar, expressed as invert sugar, in the original mixture. When this sum is divided by the percentage of total sugars, expressed as invert sugar, in the finished preserve, the quotient multiplied by 100 will be the pounds of finished preserve obtained from the original fruit and sugar mixture. The difference between this figure and the weight in pounds of the fruit and sugar used in the original mixture will be the amount of added water in the finished preserve.

For example, if the proportion of fruit to sugar as found by the procedure previously described is 14 pounds of fruit to each 55 pounds of sugar and the average content of sugar in the fruit is 6 per cent, then the pounds of sugar in the fruit will be 0.84 (6 per cent of 14). In the process of cooking, the 55 pounds of added sugar in the original mixture will be inverted to a greater or less extent. Regardless of the degree of inversion, conclusions will be accurate if both the sugar in the original mixture and the sugar in the preserve are compared on the basis of complete inversion. When 55 pounds of sugar is completely inverted, it will weigh 57.9 pounds. The sum of the sugar in the fruit and the added sugar is 58.74 pounds (0.84 lb. plus 57.9 lbs.). If the total sugars after inversion in the preserve are 67 per cent, as determined by analysis, the weight of the finished preserve will be 87.7 pounds $\left(\frac{58.74}{67}\times 100\right)$. The difference between the weight of the finished preserve, 87.7, and the weight of the original fruit and sugar mixture, 69 (14 plus 55), will be 18.7, which is the pounds of added water present in 87.7 pounds of preserve, or 21 per cent of added water.

SUMMARY

The procedure used at the present time by the Food and Drug Administration in determining the fruit content of market samples of alleged preserves and jams from their analysis, is outlined, and its application is demonstrated on products of known composition.

ACKNOWLEDGMENT

The collection of the authentic fruit data summarized herein and the study of their use in interpreting chemical analyses of preserves were begun under the leadership of H. J. Wichmann, L. H. Chernoff, and R. W. Hilts (deceased). They were succeeded by C. P. Lathrop and V. B. Bonney, who immediately supervised the work until about 8 years ago, when the project was assigned to the writer. The analysts in the various

laboratories of the Food and Drug Administration that are actively engaged in the analysis of preserves are responsible for the details of laboratory technic described.

Note on the Extraction Method for Santonin in Mixtures*

The following method has been found convenient for the direct gravimetric determination of santonin in liquid preparations containing emodin and other plant extractives, and it can be applied to other mixtures.

Transfer the accurately measured or weighed portion of sample to a suitable separator, make slightly acid with HCl, and extract with portions of $CHCl_3$ to remove all the santonin. If necessary, evaporate the $CHCl_3$ to about 100 cc. and shake thoroughly with 20 cc. of 10% NaOH in a separator. Draw off the $CHCl_3$ and wash with 20 cc. of water. Filter the $CHCl_3$ through cotton or filter paper into a tared beaker. Use 25 cc. of $CHCl_3$ to wash the NaOH solution and water, and add to the major portion of $CHCl_3$. Evaporate the $CHCl_3$, dry at 100°C. for 30 minutes, and weigh the residue. Determine the melting point of the residue to ascertain whether it is pure santonin.

(Further purification may be made by treating the residue of santonin with $Ba(OH)_2$ solution, filtering, and re-extracting the santonin after acidification. Neutral substances or oils can be removed by extracting the alkaline solution of santonin with $CHCl_3$ before acidification.)

Advantage is taken of the rather stable lactone group in santonin to separate it from most of the other acidic, resinous, phenolic, or alkaloidal compounds.

When in a chloroform solution, santonin is not affected by 10 per cent sodium hydroxide solution. The dry santonin, however, is hydrolyzed by sodium hydroxide or by barium hydroxide to form water-soluble salts, which upon acidification re-form santonin.

Chloroform will extract santonin quantitatively from acidified solutions containing as much as 50 per cent glycerol.

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* By Irwin S. Shupe, U. S. Food and Drug Administration, St. Louis, Mo Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C , November 1, 2, and 3,1937.

BOOK REVIEWS

The Soybean Industry. By A. A. HORVATH, Chemist, Agricultural Experiment Station, Newark, Delaware. v+197 pp. The Chemical Publishing Company of New York, Inc. 1938. Price $4.00.

Despite the publisher's claim that it is "Invaluable to technical workers as well as dealers and executives in the soybean industry," this volume will more than likely prove to be a disappointment to those who must daily grapple with the problems of production and utilization of the soybean and its derived products. Far from being "an authoritative concise book," it appears even to the casual reader to consist of a descriptive melange of Manchurian, European, and American practices together with condensed and uncritical reviews of the extant literature and patents. Descriptions of oil mill and refinery operations are sketchy to the point of being non-informative, often times inaccurate, and in not a few instances relate to processes almost if not wholly obsolete so far as American practice is concerned.

Due in part no doubt to the secrecy that enshrouds many of the industrial operations, little information is presented concerning the methods by which the processing of soybeans is carried out or how the derived products are utilized by the consuming industries. For example, the chapter entitled "Refining of Soybean Oil" is devoid of any description of the various operations of a modern refinery. No mention is made of the present method of continuous centrifugal refining or the recent introduction of continuous deodorization. In fact the only information on the subject of refining consists of a quotation from the official methods of the American Oil Chemists' Society for the laboratory determination of refining loss. Refinery operators and oil technologists will be surprised to learn (p. 85) that, "The minimum refining losses amount to about 10 percent of the original crude oil," that the optimum temperature of deodorization "is 150–155°C.," and that reversion "is not due to oxidation . . . due to the presence of some antioxidant" (p. 89). The pro-oxidant, carotene, and the inactive sterols are referred to as "antioxidants" (p. 90).

The chapter entitled "Soybean Oil" contains but a single analysis, actually the only one in the entire book, of a soybean oil, and it is quoted from Jamieson's "Vegetable Fats and Oils." The drying oil industry, which annually consumes over 17 million pounds of soybean oil, is dismissed in 5½ pages, whereas the subject of phosphatides, although economically and industrially of only minor importance, receives 28 pages of treatment.

Similarly, the industrial processing of the oil is disposed of in 32 pages, of which but 2½ pages are devoted to the hydrogenation industry, which consumes 70 per-cent of the total oil production. Most of the discussion of hydrogenation has reference to laboratory experiments, the practical value of which may be judged from the statement (p. 96), "It was recently found that with soybean oil the most effective catalyst was unreduced copper-nickel with 70 per cent copper and 30 per cent nickel." In contrast to the curtailed treatment of the oil, industrial utilization of the meal, at present a minor use, receives 44 pages of discussion. More than half of this space is devoted to a recapitulation of Satow's work on the glutinization process for the production of molding compounds, a process of no industrial importance.

Among the omissions of the book may be mentioned the lack of any adequate treatment of the sterols and pigments and of reference to the excellent work of T. P. Hilditch in England on the composition of soybean oil, phosphatides, and antioxidants. Furthermore, the book, despite its title, does not contain a single flow sheet or illustration of any of the numerous processes which taken as a whole comprise the soybean processing industry. The bibliography is neither extensive nor

critical. The impression that the book has been compiled from the literature rather than from a first-hand knowledge of the soybean industry can easily be gained by the reader.—K. S. MARKLEY.

Drugs and Galenicals:—Their Quantitative Analysis. By D. C. GARRATT, with a foreword by Sir Frederick Menzies. 422 pages. John Wiley & Sons, Inc., New York, 1937. Price $6.00.

The procedures required for the analysis of medicinal preparations are so varied, the methods so numerous and complex, and the pertinent information is so widely scattered in the literature, that no single book or compilation may be expected to cover the entire subject adequately.

It has been 18 years since Fuller's book, "Chemistry and Analysis of Drugs and Medicines," was published in this country, and no revision of it has appeared. During this interval numerous new drugs have been introduced, many improvements in analytical procedures elaborated, and entirely new methods devised, yet no book has appeared to give adequately both qualitative and quantitative tests for drugs in unknown mixtures. This book is confined to the simpler field of quantitative analysis of preparations of known composition.

The aim has been to gather the best methods from the literature, critically review the procedures if deemed necessary, and present them in a condensed form. That some of the later approved methods have been overlooked is not surprising when the magnitude of the task is considered. Most methods are given sufficiently in detail so that reference to the original literature is not necessary. However, many procedures are described in bare outline, from which it is assumed, of course, that the book is to be used only by skilled analysts.

The subject matter is arranged alphabetically (acetanilid to zinc), but additional sections are provided on "Oils, Fats and Waxes" and "Essential Oils." Also short treatises are included in the appendix on such topics as the determination of alkaloids and alcohol, breaking emulsions, the Stas-Otto process, etc.

A particularly valuable feature of the book is represented by the numerous brief comments on the limits of accuracy and general dependability of the methods cited. In most instances these are based on the author's experience or on his analytical judgment, although criticisms by other analysts are frequently quoted; for example, on p. 65, Garratt states, in connection with the phenylhydrazine method for the assay of camphor: " . . . but the precise degree of accuracy attainable in the fundamental reactions remains to be determined." Concerning the determination of sulfur in sulfur ointment the author comments: "A method of oxidation using nitric acid and bromine, since adopted by the U. S. P., has been shown to give high results unless nitric acid is removed before precipitation" (p. 287). Again: "The decision of the authorities (of the B. P. Committee of Revision) to substitute total percolation processes in all alkaloidal determinations on the crude drug in place of aliquot part methods has eliminated a grave source of error in the assays" (p. 229).

In many instances one or more alternative procedures are given for the determination of the same constituent, the author's choice usually being indicated and the reason therefor.

The analytical results that may be expected from standard preparations are occasionally given, a feature particularly helpful to the less experienced analysts. Although this applies to British preparations, such information should be useful to American analysts as well. It is to be hoped that this policy will be extended in future editions. Topics that are particularly well covered are belladonna, pyrethrum, and the compounds of arsenic, iodine, lead, and zinc; less adequately are ipecac,

male fern, and santonica. Although the book is intended for use in Great Britain and its Possessions, drug analysts in other countries will find it useful. In the opinion of the reviewer it should be in all laboratories where drugs are analyzed.—L. E. WARREN.

Elementary Principles of Qualitative Analysis. By T. R. HOGNESS and WARREN C. JOHNSON, Associate Professors of Chemistry, University of Chicago. x+325 pp., 27 figures. Henry Holt and Co. 1938. Price $1.40.

This book is an adaptation of the authors' earlier book "Qualitative Analysis and Chemical Equilibrium" to the usual course in General Chemistry. The first 199 pages are practically identical with the first 199 pages of the earlier work. The laboratory procedure is essentially the same, but is more limited, including only the cations: ammonium, sodium, potassium, silver, lead, mercury (-ous and -ic), copper, tin, zinc, manganese, iron, chromium, aluminum, calcium, and barium, and the anions: chloride, nitrate, sulfate, carbonate, and sulfide.

The theoretical part is divided into nine sections entitled: Some General Properties of Solids, Liquids, and Solutions; Reaction Velocity and Chemical Equilibrium; Equilibria Involving Weak Acids and Bases; Heterogeneous Equilibrium—The Solubility Product—Colloids; Polybasic Acids—Precipitation with Hydrogen Sulfide; Equilibria Involving Complex Ions; Amphoteric Substances; and Oxidation and Reduction. Problems and review questions in abundance are given following each chapter. In the appendix 14 pages are devoted to an excellent discussion of the mathematical operations needed for the solution of the problems.

The book is well set up, easy to read, and neat in appearance. On the whole it is well written, and the topics are well selected for the purpose specified. The reviewer feels, however, that the Bronsted theory of acids and bases merits inclusion in such a work, and that some mention of the deviation of the "Solubility Product" from constancy at high ionic strengths should be made.

The reviewer is frankly puzzled as to where this text should fit in the chemistry curriculum. Two hundred pages of theory of equilibrium is a great deal to add to an already heavily loaded general chemistry course, while the very limited number of ions included in the qualitative scheme should preclude its use in a course in qualitative analysis.—B. D. VAN EVERA.

SECOND DAY

TUESDAY—MORNING AND AFTERNOON SESSIONS—Continued

REPORT ON DRUGS

By L. E. Warren (U. S. F ..d and Drug Administration, Washington, D. C.), *Referee*

Since the last meeting there have been two occurrences which are of interest to drug analysts:

(1) The first supplement of the U.S.P. XI appeared in August. It becomes official December 1, 1937. This is a brochure of 104 pages. It contains no strictly new topics, although permission is granted to use water other than distilled water in the manufacture of certain products.

(2) The second list of corrections for the National Formulary VI was issued in early summer. To date there has been no interim revision of the National Formulary VI, but there is a possibility that a new edition will be published about 1940.

Last year 26 topics were assigned to associate referees and another topic was added after the meeting. Substantial progress has been reported in 24 subjects. From these reports the Referee is able to recommend for adoption chemical methods for the determination of five substances, viz., cinchophen in presence of salicylates, homatropine in tablets, pyridium, and caffeine and potassium bromide in effervescent mixtures. Further, he is able to recommend the adoption of tentative microchemical methods for the identification of seven substances; viz., apomorphine, ethylmorphine, benzylmorphine, hydrastinine, benzoic acid, salicylic acid, and acetylsalicylic acid.

Last year attention was called to the fact that the Association is now studying directions for preparing and standardizing certain volumetric solutions. The Association has a referee on the subject. Although all of the volumetric solutions described in *Methods of Analysis* do not come within the purview of the Drug Section, many do, and such information is naturally of much interest to drug analysts. Among the solutions so prepared and standardized are sodium hydroxide, hydrochloric acid, and alcoholic potassium hydroxide, aldehyde free. Undoubtedly standardization methods for other volumetric solutions will be adopted from time to time.

Microchemical Tests for Alkaloids.—Previous to this year the Association had adopted one or more microchemical methods for the identification of 29 alkaloids, including a few synthetics having alkaloidal properties. This year apomorphine, hydrastinine, ethylmorphine (dionine), and benzylmorphine (peronine) were studied. Last year, *This Journal*, **20**,

55 (1937), the Association recommended that berberine and cytisine be studied also. Supplies of cytisine could not be obtained, and the preliminary tests on berberine were not sufficiently good to warrant collaborative tests. The observation by Shupe that apomorphine hydrochloride is precipitated by hydrochloric acid was confirmed, but it was found that benzylmorphine also reacted in the same way. The microchemical tests submitted to the collaborators gave satisfactory identifications. The associate referee recommends that the microchemical tests for hydrastinine, ethylmorphine (dionine), benzylmorphine (peronine), and apomorphine be adopted as tentative. He further recommends that the topic be continued to include berberine, coniine, narcotine, and narceine; also cytisine if available. The Referee concurs.

Microchemical Tests for Synthetics.—Previous to this year the Association had adopted microchemical methods for the identification of 15 synthetic substances in addition to several synthetics that have alkaloidal properties. The subject of microchemical tests for synthetics is such a large one that it may be said that the Association has only just started in its studies of this topic. This year microchemical methods for acetylsalicylic acid, benzoic acid, and salicylic acid were studied. No report was submitted on diallyl barbituric acid which Subcommittee B recommended for study last year. The associate referee recommends that the microchemical methods for each of the substances that were studied collaboratively be adopted as tentative. This is approved. The associate referee further recommends that "other important synthetics be studied." The Referee recommends that sulfanilamide, mandelic acid, and diallyl barbituric acid be studied.

Hypophosphites.—The bromine oxidation method was studied by the associate referee this year. Good results were obtained in the analysis of commercial sirup of hypophosphites but no collaborative work was done and no samples of known composition were prepared and assayed. The associate referee recommends that the bromine oxidation method be subjected to collaborative study. The Referee concurs.

Santonin, Phenolphthalein, and Calomel in Tablets.—Last year the collaborative results for the determination of santonin and calomel were good, but several collaborators reported high values for phenolphthalein. This year the work was confined to the separation and determination of phenolphthalein in such mixtures. The results were somewhat erratic. The associate referee recommends continuation of the topic. This is approved.

Daphnia Methods.—The associate referee has continued his studies on the application of daphnia to the evaluation of various drugs such as laxatives, pilocarpine, strychnine, and certain vitamins. He recommends that the topic be continued. The Referee concurs.

Determination of Hexylresorcinol in Olive Oil Solutions.—A tentative method for the determination of hexylresorcinol was adopted last year,

This Journal, **20**, 81 (1937). The topic was continued to include determination of the substance in preparations. The associate referee has developed a method in which oxidation is prevented by hydrazine. The associate referee and two collaborators obtained good results in assays of one known specimen of hexylresorcinol in olive oil. The associate referee recommends that the subject be continued in order to obtain more collaborative results and to extend the method to other preparations containing hexylresorcinol. The Referee concurs.

Ergot Alkaloids.—This topic has been studied for several years. Last year the total alkaloids were extracted in an automatic extractor; this year by the hand separator. The latter method does not secure complete extraction. Since the problem appears to be in its preliminary stages the associate referee recommends its continuance. In this the Referee concurs.

Nitroglycerin in Mixtures.—The associate referee continued his work of last year and reports the results from two collaborators as well as his own findings. The double distillation method gave consistent results on tablets (whole and powdered), but in complex mixtures the results were low. The Referee recommends that the subject be continued.

Guaiacol.—The associate referee devised a method for the determination of guaiacol that depends on the determination of the methoxy group. He applied the method to guaiacol and to guaiacol carbonate and found it satisfactory. However, he has been unable to submit the method to collaborative study. The Referee recommends that the subject be continued.

Biological Testing.—The associate referee reports that no work was done. It is recommended that the topic be continued.

Iodine Ointment.—The associate referee and his collaborators tried methods for the determination of free iodine and for organically combined iodine. The method for free iodine was found satisfactory and is recommended for tentative adoption. The method for organically combined iodine was not satisfactory. The associate referee recommends that the subject be continued. These recommendations are approved.

Aspirin, Acetophenetidin, and Caffeine in Mixtures.—This topic has been studied for several years. According to a preliminary report, this year the associate referee devoted his energies particularly to the separation and recovery of caffeine, but no method was developed sufficiently well to warrant collaborative work. The associate referee's recommendation that the subject be continued is approved.

Pyridium.—The associate referee worked out a method for assaying pyridium (base) in jellies, ointments, and tablets. The method is an application of the titanium chloride titration for certain dyes. Good results were obtained by the associate referee on knowns, but little collaborative work was done. The recommendation of the associate referee that the proposed method be studied collaboratively is approved by the Referee.

Gums.—Methods for the identification of gums, either singly or in mixtures, have been studied for three years but none has been adopted. Methods developed for the identification of single gums were moderately satisfactory, but they were totally inadequate when applied to mixtures. Furthermore, the reactions observed for pure commercial gums (unmixed with other substances) did not correspond with those obtained by the residues after the gum had been separated from pharmaceuticals. This year no work was done. The Referee is of the opinion that the topic should be continued.

Cinchophen in Presence of Salicylates.—The associate referee applied the well-known Emery method to this problem. The results obtained by each of the collaborators were good. The associate referee recommends that the method subjected to collaborative study be adopted as tentative and that the subject be closed. The Referee concurs.

Theobromine Calcium Tablets.—The Association has adopted a periodide method for the assay of theobromine in theobromine calcium. This year the associate referee and his collaborators studied theobromine calcium tablets. (The method used is an adaptation of the silver nitrate procedure of Boie.) They also applied the method to pure theobromine and to theobromine calcium powder. The results are in good agreement. The associate referee recommends that the Boie method be adopted as tentative to supersede the periodide method already adopted. This recommendation applies to theobromine also. The Referee is of the opinion that the Boie method should be adopted as an alternative tentative procedure, but that the adoption of the periodide method should not be rescinded, and he so recommends. The associate referee recommends that an application of the method to theophylline be studied. The Referee doubts the wisdom of this action at this time since the U.S.P. XI and the A.O.A.C. have each adopted a method for the assay of theophylline.

Chlorbutanol.—The associate referee has applied a distillation method to products containing known amounts of chlorbutanol, with subsequent conversion of the chlorine to chloride by alcoholic potassium hydroxide in a pressure bottle. The collaborative results were not entirely consistent. The associate referee recommends that the subject be continued. The Referee concurs.

Aspirin and Phenolphthalein Mixtures.—This subject has been under consideration for three years. Last year the associate referee applied the Hitchens method, *J. Am. Pharm. Assoc.*, **23**, 1084 (1934), to a mixture of phenolphthalein, acetylsalicylic acid, and starch. His results were promising, but the findings by the collaborators were disappointing. This year the collaborative results were still unsatisfactory. The recommendation of the associate referee that the subject be continued is approved.

Homatropine in Tablets.—This subject has been studied for two years. Last year the associate referee found that the tentative A.O.A.C. method

for atropine in tablets, *Methods of Analysis*, 1935, 555, gave reasonably good results for homatropine, but no collaborative work was done. This year the method was subjected to collaborative study with good results. The associate referee recommends the method for adoption as official, first action. Although it is somewhat unusual to adopt a method as "official, first action" without the method having passed through the tentative adoption stage, it may be justifiable in this case since the method is a standard one for other alkaloids and since it gave good results in the hands of the associate referee last year. The Referee concurs with the recommendation of the associate referee.

The associate referee has devoted a great deal of time to the search for a method of separating homatropine from cocaine but without success. He recommends that the subject be closed. The Referee concurs.

Cubeb.—An assay method for cubeb has been studied for two years. The method used is an adaptation of the method for volatile oils in spices, *Methods of Analysis*, 1935, 447. Last year the National Formulary adopted an assay method for cubeb. This is a determination of "Volatile Ether Extract" and differs greatly from the A.O.A.C. method.

Since the N.F. VI method and the A.O.A.C. method are different in principle, it was decided last year to continue work on cubeb. The associate referee reports good results with the method sent out for collaborative study. His recommendation that his method be adopted as tentative for cubeb is approved. He further recommends that the subject of cubeb be closed and that cardamom be studied. Both of these recommendations are approved.

Aminopyrine and Phenobarbital in Mixtures.—The associate referee devised a somewhat empirical method of separation by which the aminopyrine is removed by chloroform from an aqueous alkaline solution. Afterward the aqueous solution is acidified and the phenobarbital is removed by ether. The results were reasonably satisfactory. It is recommended that the subject be continued to include collaborative work.

Effervescent Potassium Bromide with Caffeine.—The associate referee and his collaborators have applied well-known analytical methods for the assay of bromide and caffeine to this product. The results are excellent. The associate referee reports that caffeine cannot be dried for long periods at 100° C. without slight losses. He recommends drying at 80° C. His recommendations, that the methods submitted to collaborative study be adopted as tentative, are approved.

Elixir of Terpin Hydrate with Codeine.—The A.O.A.C. has adopted a method for the assay of elixir of terpin hydrate but none for codeine, if present in the elixir.

The associate referee and his collaborators have used a method for the determination of codeine, which was elaborated by several other Association chemists. The results for codeine were reasonably good but for terpin

hydrate only fair. The recommendations of the associate referee that the method used be adopted as tentative for codeine in elixir of terpin hydrate and codeine is approved.

Emulsions of Cod-liver Oil.—Various methods of extracting the oil from emulsions were tried by the associate referee. These included shaking out with chloroform in a separator, and extraction with chloroform in a Soxhlet apparatus. Neither the Mojonnier tube nor the Roese-Gottlieb method in a Röhrig-Biesterfeld tube (*J. Ind. Eng. Chem.*, **9**, 1111 (1917); *Methods of Analysis, A.O.A.C.*, 1935, 267) was tried. The Referee has used these methods on emulsions of liquid petrolatum with satisfactory results.

The associate referee recommends that the topic be continued. The Referee concurs.

Ointment of Mercuric Nitrate (*Citrine Ointment*).—This is a new topic. The associate referee has applied a modification of the thiocynate method to this product. The results, in general, were somewhat low. The Referee suggests that this may be due to insufficient melting and washing. The method, as sent to the Referee for collaborative study, made but one melting of the fatty material. The associate referee is aware that his proposed method gives low results, but he is of the opinion that, since citrine ointment is a subject of small importance in pharmacy, the topic should be closed.

Since so much work has already been done, the Referee is of the opinion that the topic should be continued for one more year.

Rhubarb and Rhaponticum.—This topic has been under consideration for a number of years. Some interesting reports have been published, but they have not resulted in the adoption by this Association of any methods for distinguishing between the two drugs. This year the associate referee attempted to secure authentic specimens of rhaponticum for study and reviewed the subject from the published data. To date he has not secured supplies but contacts with importers are in progress. His recommendation that the topic be continued is approved.

Theophylline Sodium Salicylate.—Last year, *This Journal,* **20,** 82 (1937), the Association adopted a method for the assay of theophylline that is applicable to most preparations of the drug such as solutions, tablets, etc. The process consists in dissolving out the alkaloid from its preparations with sodium hydroxide, acidifying the solution, and shaking out with a mixture of chloroform and isopropyl alcohol. The method was not tried on theophylline sodium salicylate for the reason that it might not be expected to work on this preparation due to the liberation of free salicylic acid during the procedure. This would be extracted and weighed as theophylline (with danger of loss due to volatility).

This year the associate referee devised a clever method of separation based on the differing solubilities of salicylic acid and theophylline in

different mixtures of solvents. He also devised a volumetric silver precipitation method for theophylline, after which the salicylic acid is determined by the official A.O.A.C. method, *Methods of Analysis*, 1935, 551. The associate referee recommends that both methods for theophylline be adopted as tentative for theophylline salicylate. He further recommends that the methods when applied to mixtures with excipients be studied further. The Referee believes that further work should be done and so recommends.

Sulfanilamide.—This compound has recently come into use as a treatment for infections with beta-hemolytic streptococci. Methods for its evaluation have been proposed but have not been considered by the Association. It is recommended that sulfanilamide be studied.

Mandelic Acid.—During the past few years mandelic acid and its salts have come into extended use in the treatment of certain infections. It is recommended that mandelic acid and its salts be studied.

Arecoline Hydrobromide.—Arecoline hydrobromide is being used considerably in veterinary medicine. No methods of assay for the product from the alkaloidal standpoint are provided in the various compendiums. It is recommended that the subject be studied.

REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS

By CHRIS K. GLYCART (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In continuing the subject this year, the Associate Referee studied tests for ethylmorphine (dionine), benzylmorphine (peronine), and hydrastinine. The single test for apomorphine with potassium iodide reagent reported last year was considered to be insufficient, *This Journal*, **20**, 551 (1937). The test with gold chloride now submitted is sensitive in solutions 1 to 10,000. Red-brown needles in dense masses are formed immediately. According to Stephenson[1] this is the best test for apomorphine. The importation of peronine is prohibited by the U. S. Treasury Department. Fortunately, some of this material was found in this laboratory, and it responded to the tests described by Stephenson and by Amelink,[2] although the specimen was 25 years old. Benzylmorphine, like apomorphine, forms a precipitate with hydrochloric acid, but the crystals are often notched at the ends.

Further study of berberine is necessary because in the preliminary work similar crystals were produced by the various reagents. A supply of cytisine was not available.

[1] Microchemical Tests for Alkaloids, 1921.
[2] Schema zur Microchemischen Identification von Alkaloiden, 1934.

Directions for the tests, control specimens consisting of hydrastinine hydrochloride N.F., ethylmorphine hydrochloride U.S.P., apomorphine hydrochloride U.S.P., and peronine, also samples for identification marked Nos. 1, 2, 3, and 4, were sent to the collaborators. The method was published in *This Journal*, **21**, 91 (1938).

RESULTS AND COMMENTS BY COLLABORATORS

Chemical Control Laboratories, Eli Lilly and Company.—(1) Hydrastinine, (2) Apomorphine, (3) Benzylmorphine (peronine), and (4) Ethylmorphine (dionine).

All these tests proved satisfactory to us. We would suggest stirring in the test for ethyl morphine (dionine) with mercuric chloride reagent.

Irwin S. Shupe, U. S. Food and Drug Administration, St. Louis, Mo.—(1) Hydrastinine, (2) Apomorphine, (3) Benzylmorphine (peronine), and (4) Ethylmorphine (dionine).

From comparisons with controls, I had no difficulty in identifying the unknowns. The crystals of apomorphine and peronine with HCl are much alike. Dionine with Wagner's reagent produced an amorphous precipitate out of which crystals formed slowly.

Kenneth L. Milstead, U. S. Food and Drug Administration, Chicago, Ill.—(1) Hydrastinine, (2) Apomorphine, (3) Benzylmorphine (peronine), and (4) Ethylmorphine (dionine)

All tests were applied to both controls and samples, and the characteristic crystals described were obtained

F. K. Ballard, Customs Laboratory, U. S. Treasury Department, Chicago, Ill.—(1) Hydrastinine, (2) Apomorphine, (3) Benzylmorphine, and (4) Ethylmorphine.

All the crystalline precipitates were obtained as described in the method. The reaction of hydrastinine with $KMnO_4$ solution appeared to be improved by using a minimum amount of the solution. The best results were obtained by using the $KMnO_4$ solution in a volume approximately one-fifth that of the solution of hydrastinine hydrochloride. On standing, the red plates gradually turned brown, apparently decomposing with formation of an oxide of manganese. Crystals were obtained with ethylmorphine hydrochloride and Wagner's reagent only on stirring, the precipitate otherwise consisting only of minute brown oil-like globules.

John R. Matchett and Joseph Levine, Bureau of Narcotics, Treasury Dept., Washington, D. C.—(1) Hydrastinine, (2) Apomorphine, (3) Benzylmorphine, and (4) Ethylmorphine.

With the known compounds, in the same form immediately at hand for comparison, the identifications were made without difficulty. In cases where an unknown alkaloid has been extracted from a solution, we are of the opinion that one would be led to the proper tentative identification in the cases listed below. It is always absolutely necessary, of course, that the known and unknown be compared side by side before a final identification may be made.

Hydrastinine with $KMnO_4$, hydrastinine with $HgCl_2$, apomorphine with HCl, benzylmorphine with KI, Benzylmorphine with NH_4CNS, benzylmorphine with HCl, ethylmorphine with $HgCl_2$, and ethylmorphine with Wagner's reagent.

The crystals formed in most cases were found to be very much as described. The following comment is offered:

1. *Apomorphine with gold chloride.*—The crystals were found to be so fine that the precipitate appears almost amorphous. They are better in very dilute solutions.
2. *Apomorphine with hydrochloric acid.*—The crystals were found to be as described. Most of the rods had notched ends.

3. *Hydrastinine with potassium permanganate.*—The 5% reagent solution is too deeply colored to permit satisfactory examination of the drop. A 0.1 N solution was found to be better than the one suggested if the alkaloid solution is more concentrated than 1:500.

4. *Hydrastinine with mercuric chloride.*—The crystals were found to be as described.

5. *Hydrastinine with potassium ferrocyanide and 5% hydrochloric acid.*—In 1:200 solution crystals formed only after long standing. With stronger solutions a number of different crystal forms were observed in the same drop. This reagent is regarded as less satisfactory than the others for hydrastinine.

6. *Ethylmorphine with Wagner's reagent.*—Good crystals formed slowly in 1:200 solution. With greater concentration of the alkaloid good crystals formed much more quickly. The form was as described.

7. *Ethylmorphine with mercuric chloride.*—Crystals as described were formed if the concentration of alkaloid was greater than 1:100.

8. *Benzylmorphine with potassium iodide.*—Crystals were found to be as described.

9. *Benzylmorphine with ammonium thiocyanate.*—Crystals were found to be as described.

10. *Benzylmorphine with hydrochloric acid.*—Crystals were found to be as described. They formed only on stirring the drop.

In all cases the concentrations given refer to alkaloid in the drop prior to addition of reagent.

A. H. Shaffer and H. C. Elliott, U. S. Bureau of Internal Revenue, Chicago, Ill.—(1) Hydrastinine, (2) Apomorphine, (3) Benzylmorphine (peronine), and (4) Ethylmorphine (dionine).

It was found that by diluting the 5% $KMnO_4$ reagent to a concentration of 1–2 per cent, the characteristic crystals formed with hydrastinine were more readily observed.

CONCLUSION

The alkaloids were identified correctly by the six collaborators. With regard to the test for hydrastinine, Matchett, Ballard, and Shaffer observed that the 5 per cent potassium permanganate reagent specified was too strong. The directions were accordingly changed to a 1 per cent reagent.

RECOMMENDATIONS[1]

It is recommended—

(1) That the microchemical tests presented for hydrastinine, ethylmorphine (dionine), benzylmorphine (peronine), and apomorphine be adopted as tentative.

(2) That the study be continued and include berberine, coniine, narcotine, and narceine, also cytisine if available.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 65 (1938).

REPORT ON MICROCHEMICAL TESTS FOR SYNTHETICS

By IRWIN S. SHUPE (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

Salicylic, benzoic, and acetylsalicylic acids are the subjects of study in this report. Microchemical methods for the identification of these substances are given by Mayrhofer,[1] making use of the crystalline silver salts. A. J. Steenhauer[2] uses copper-pyridine complex for identifying certain organic acids.

A preliminary investigation of the characteristics of the silver and copper reagents showed them to be quite general for aromatic organic acids. In an attempt to find more selective reagents, tests were made with triethanolamine, pyridine, cyanide, and ammonium complexes of the following metals: zinc, nickel, cobalt, copper, cadmium, silver, mercury, lead, and iron. Alkaline complexes were used because of the relative insolubility of the organic acids in water or acid aqueous solutions.

Lead triethanolamine and zinc-pyridine complexes were found to yield crystalline precipitates with benzoic and salicylic acids and are considered more specific than copper or silver. The silver salt of acetylsalicylic acid has a characteristic structure, however, and with experience can be used to differentiate benzoic and salicylic acids. Triethanolamine is superior to ammonia as a solvent because it does not hydrolyze acetylsalicylic acid so readily. Bromine solution distinguishes salicylic acid from benzoic or acetylsalicylic acid.

Unknown samples sent to collaborators for identification were (1) acetylsalicylic acid, (2) benzoic acid, and (3) salicylic acid.

The methods were published in *This Journal*, **21**, 93 (1938).

RESULTS AND COMMENTS BY COLLABORATORS

The methods for identification and the unknown were sent to collaborators, all members of the U. S. Food and Drug Administration, who reported as follows:

Samuel Alfend, St. Louis, Mo.—I. Acetylsalicylic acid, II. Benzoic acid, and III. Salicylic acid.

Salicylic acid: $AgNO_3$ test (1:100) mostly rods not easily identified; with weaker (1:150 or 1:200) mostly plates and crosses more easily identified.

Benzoic acid: First 2 tests unsatisfactory.

Kenneth L. Milstead, Chicago, Ill.—I. Acetylsalicylic acid, II. Benzoic acid, and III. Salicylic acid.

I found no particular difficulty in applying these tests.

E. C. Deal, New Orleans, La.—

Reagents	*Acetylsalicylic Acid*	*Salicylic Acid*	*Benzoic Acid*
Silver nitrate	I	III	II
Bromine solution		III	
Lead triethanolamine		III	II
Zinc pyridine			II

[1] Mikrochemie der Arzneimittel und Gifte, pp. 210, 225 (1928).
[2] *Pharm. Weekblad*, **72**, 667 (1935); *C.A.*, 5385 (1935).

Crystal formations were very definite and characteristic, with the exception of the use of lead triethanolamine with salicylic acid. Here there was a tendency to form an amorphous precipitate.

Morris L. Yakowitz, San Francisco, Calif.—I. Acetylsalicylic acid, II. Benzoic acid, and III. Salicylic acid.

All the tests except the zinc pyridine test for benzoic acid are excellent. Prismatic crystals formed in about 20 minutes.

Paul S. Jorgensen, San Francisco, Calif.—I. Acetylsalicylic acid, II. Benzoic acid, and III. Salicylic acid.

No difficulty was experienced, and the tests appear to be satisfactory.

DISCUSSION

Tests in which the dry powdered substance is added directly to a drop of the reagent are convenient and save time but they lack precision and uniformity due to variations in stirring and in the amounts of powdered material used. Some of the difficulties reported by collaborators are probably due to these variations in technic. In the tests for benzoic acid with zinc-pyridine and lead-triethanolamine, it is necessary to stir to induce crystallization. Crystals begin to form on the glass slide where it has been rubbed. The caution "Stir thoroly to induce crystallization" was added to the directions. The methods as modified are considered to be suitable for the identification of these synthetics.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods presented for identification of benzoic, salicylic, and acetylsalicylic acids be adopted as tentative.

(2) That other important synthetics be studied.

REPORT ON HYPOPHOSPHITES

By HENRY R. BOND (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The quantitative determination of hypophosphites in sirup preparations by bromine oxidation was studied this year, but no time was available to the Associate Referee for the preparation of samples for collaborative study.

The method of assay that was studied briefly is one devised by C. F. Bruening,[2] a modification of his method of assay as applied to the simple salts. Material assayed consisted of three sirups prescribed in the National Formulary VI: (1) Sirup Ammonium Hypophosphites, (2) Sirup Hypophosphites, and (3) Sirup Hypophosphites Compound, all products of the same reputable manufacturer. Samples 2 and 3 were selected because of their somewhat complex nature.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 65 (1938).
[2] *J. Am. Pharm. Assoc.*, 25, 6 (1936).

The following reagents and procedure were used:

REAGENTS

Bromide-bromate solution.—0.1 *N*. Dissolve 3 grams of $KBrO_3$ and 50 grams of KBr in water and dilute to 1 liter.

Sodium thiosulfate.—0.1 *N*.

Potassium iodide.—20 grams per 100 cc.

Sulfuric acid.—10 grams per 100 cc.

Starch solution.—0.5 gram per 100 cc.

PROCEDURE

Transfer exactly 50 cc. of the sirup, measured in a volumetric flask, to a 250 cc. volumetric flask. Wash the flask with several portions of distilled water, adding the washings to the 250 cc. flask, and finally make up to the mark with distilled water. (This dilution was used for the Sirup Ammonium Hypophosphite. For the other two sirups the original 50 cc. was diluted to 500 cc. in a volumetric flask.) In all cases transfer a 50 cc. aliquot to a 250 cc. volumetric flask and make up to the mark with distilled water. Transfer a 50 cc. aliquot of this solution to a 500 cc. glass-stoppered Erlenmeyer flask, add 50 cc. of the bromide-bromate solution and 20 cc. of the H_2SO_4; stopper, shake well, and allow to stand for 2 hours. Add 10 cc. of the KI solution, shake the flask, and titrate the liberated I with the $Na_2S_2O_3$ until the solution becomes straw colored, then add 2 cc. of the starch solution and titrate until the solution becomes colorless. Carry out a blank determination in the same way. The difference between the two titrations with the $Na_2S_2O_3$ multiplied by the appropriate factor gives the amount of hypophosphite salt present.

The results in terms of grams of hypophosphorous acid per 100 cc. and their percentage relationship to the calculated theoretical amount of H_3PO_2 in grams per 100 cc. are shown in the table.

SAMPLE	ALIQUOT	AMMONIUM HYPOPHOSPHITE		HYPOPHOSPHITES		HYPOPHOSPHITES COMPOUND	
		grams H_3PO_2 per 100 cc.	*per cent*	*grams H_3PO_2 per 100 cc.*	*per cent*	*grams H_3PO_2 per 100 cc.*	*per cent*
A	1	2.896	102.91	5.035	100.45	5.567	102.90
	2	2.896	102.91	5.002	99.80	5.534	102.29
B	1	2.892	102.77	5.035	100.45	5.517	101.98
	2	2.900	103.05	5.035	100.45	5.509	101.83
Calc'd. theoretical		2.814		5.012		5.532	
Av.			102.91		100.29		102.29

DISCUSSION

The results, although based on brief experiment, indicate that the rapid volumetric method devised by Bruening merits further consideration. Such consideration must involve a study of the effects of the other ingredients of the sirups (sucrose, glycerol, citrate, etc.) upon the bromine reagent. The slightly high results obtained tend to indicate the probability of action of these ingredients. However, application of the method to authentic sirups made from ingredients assayed gravimetrically prior

to their incorporations in the sirups, may produce results in closer conformity to the theoretical than those obtained through assay of the commercial products used.

It is recommended[1] that the Bruening method of assay of sirups containing hypophosphites be subjected to extensive collaborative study.

REPORT ON SANTONIN, PHENOLPHTHALEIN, AND CALOMEL IN TABLETS

By HARRY J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

Last year a method for the determination of santonin, phenolphthalein, and calomel in mixtures was submitted to collaborative study, *This Journal*, **20**, 558 (1937). Results for santonin and calomel were excellent, but four of the six collaborators reported high results for phenolphthalein. Since the method was satisfactory for santonin and calomel, the Associate Referee confined his study this year to the phenolphthalein determination.

In the report of Subcommittee B, *Ibid.*, 55, it was suggested that the cause of the high results might be the inexperience of some collaborators with the iodine method. A study of the reports[2] on the basis of which the iodine method was originally adopted shows, however, that the method tends to give low instead of high results when directions are not carefully followed. The Associate Referee believes, therefore, that the high results of the collaborators were due to contamination of the tetraiodophenolphthalein with some other constituent of the mixture, probably santonin.

Reassay by the Associate Referee of last year's sample gave figures for phenolphthalein of 16.43 and 16.48 per cent. It is evident that the technic of the Associate Referee differs in some respect from that of the collaborators obtaining high results. A consideration of the chemical properties of santonin led to a theory as to where the difference lay. Santonin is practically insoluble in water, but, being a lactone, it will react with alkalies to form santoninic acid, whose salts are water-soluble. Acidification of an alkaline santoninate reforms santoninic acid, which in the presence of strong acids quickly reverts to the water-insoluble santonin. In the proposed method the phenolphthalein is dissolved in strong (1+1) potassium hydroxide with the addition of more or less water. When the reaction with iodine is completed, the alkaline tetraiodophenolphthalein solution is filtered. The tetraiodophenolphthalein is finally precipitated, filtered, dried, and weighed. What should happen to santonin under these conditions? If the exposure of the santonin to alkali were not sufficient

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 65 (1938)
[2] Palkin, *J. Ind. Eng Chem*, **12**, 766 (1920); *This Journal*, **7**, 14 (1923–24); **8**, 30 (1924–25)

for the alkali to hydrolyze the santonin to santoninic acid, the santonin would remain undissolved and would be filtered off before the final precipitation of the tetraiodophenolphthalein. In this case the method should give correct results for phenolphthalein. On the other hand, if the reaction of alkali with the santonin were complete, all the santonin would be in solution at the start of the iodination. Upon each acidification more or less of the santonin would be precipitated, either as such or as santoninic acid. (The tendency of santonin to form supersaturated solutions enters here.) If all the santonin were dissolved the last time the tetraiodophenolphthalein was dissolved in alkali, and all of it were precipitated on the final acidification, it would all (disregarding its solubility in hot water) be weighed with the tetraiodophenolphthalein. The figures of the collaborators for phenolphthalein, while high, show that only a small proportion of the santonin is carried through. It would appear reasonable to believe that at some point in the collaborators' determinations some of the santonin was dissolved by the alkali and was not finally reprecipitated until the final precipitation of the tetraiodophenolphthalein.

In the belief that the first solution of the phenolphthalein in alkali, where both heat and strong alkali might have been used by some collaborators, offered the most likely place in the method for santonin to be dissolved, the Associate Referee rewrote the method, covering this point and emphasizing the necessity of filtering the alkaline tetraiodophenolphthalein solution. A sample identical in composition with that of last year was sent to all collaborators who had worked on the previous sample. It was requested that it be analyzed as follows:

Weigh 4 grams into a Caldwell crucible and wash with about 200 cc. of hot alcohol, collecting the filtrate in a 250 cc. volumetric flask, using a bell-jar and suction. Make the filtrate to volume at 20° C. Evaporate a 50 cc. aliquot to dryness, and proceed as directed in *Methods of Analysis*, 1935, p. 570, **80**. Do not use heat in dissolving the residue in KOH solution and water. Part of the residue will not go into solution; disregard this and proceed with the iodination when it is evident that all the phenolphthalein is dissolved. Filter the cold solution of tetraiodophenolphthalein according to directions, even though there may be no visible insoluble matter present.

Three collaborators reported. Their results (Table 1) indicate that the method still gives high values with some analysts. The most disappointing feature of the reports is that one collaborator who reported correct results last year obtained high values for phenolphthalein this year.

In correspondence, Collaborator Shupe has suggested that the length of time the tetraiodophenolphthalein solution is allowed to stand before being filtered and the amount of excess alkali present may be the factors controlling contamination of the final tetraiodophenolphthalein precipitate with santonin. In any case, the results of the two years' collaborative study of the method have forced upon the Associate Referee the belief

TABLE 1.—*Santonin-phenolphthalein-calomel mixture—1937*
(Phenolphthalein 16.70 per cent)

COLLABORATOR	PHENOLPHTHALEIN FOUND					
	1	2	3	4	AVERAGE	AVERAGE OF 1936 RESULTS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H. W. Burlage, University of North Carolina, Chapel Hill, N. C.	16.22	17.11	16.45	16.07	16.46	20.75
W. F. Reindollar, State Dept. of Health, Baltimore, Md.	17.77	17.72	18.06	17.25	17.70	17.72
I. S. Shupe, U. S. Food and Drug Adm., St. Louis, Mo.	17.3	17.4	17.2		17 3	16.7
H. J. Fisher	16.81	16.67			16.74	16 53
Average, all collaborators					17.05	17.87

that the method as written is subject to factors difficult to control with any certainty and therefore is not suitable for adoption as an official method. The safest remedy will probably lie in some method of removing the santonin before attempting to precipitate the phenolphthalein. In this connection the observation of Shupe (private communication) that santonin, *when in solution in chloroform,* is not acted on by 10 per cent sodium hydroxide, may be of value.

RECOMMENDATION[1]

It is recommended that the study of the determination of phenolphthalein in the presence of santonin and calomel be continued.

REPORT ON DAPHNIA METHODS

By ARNO VIEHOEVER (Philadelphia College of Pharmacy and Science, Philadelphia, Pa.), *Associate Referee*

The main features of this report are the exhibits and demonstrations of daphnia, and of apparatus and methods developed for routine and research work with daphnia.

The outstanding aim is to indicate the progress that has been made and to illustrate the present status of efforts of standardizing:

1. The breeding and handling of daphnia.
2. The methods of administering drugs, and the observation and the recording of their effects.

[1] For report of Subcommittee B and action by the Association, see *This Journal,* 21, 65 (1938).

I. *Food mixtures for fodder organisms*

a. Soybean ("Cellu") and urea.
b. Shredded ("Whizzard") sheep ("Boving") cow manure.

These are used in concentrations as discussed in last year's report, now in print (*This Journal*, **20**, 562 (1937).

II. *Demonstration of daphnia with micro-projector*

A. In double chambers—showing: a. Normal heartbeat
(1.5 mm. deep) b. Normal respiratory rate
c. Depressed through trional

B. In double chambers or in single (1.5 mm. deep), showing: drug effects on nervous, digestive, and muscular systems.

III. *Demonstration in narrow jars*

1. Comparative effect of strychnine: Immediate effect→convulsion at surface.
2. Comparative effect of strychnine: Time effect→convulsion at bottom.
3. Comparative effect of pilocarpine: Immediate effect→tremor.

IV. *Exhibit*

Comparoscope for rapid and time reactions.

V. *Exhibit*

Standardized breeding jars for:

1. Mass cultures (1 gallon preserve jars)
2. Controlled clutch cultures, containing 500 cc.
3. Controlled individual cultures, containing 1 oz.

VI. *Exhibit and demonstration*

Standardized observation chambers, 0.25, 0.05, 0.16 cc., and tubes, 1½, 3 cc.

VII. *Selected optics for observation*

1. 6×, 8×, 10× for direct observation.
2. 2×, 6×, 10× for projection.
3. 20–100–1000× for microscopical observation.

VIII. *Demonstration and exhibit*

Administration of medicinal agents—

1. Through culture medium—in solution or fine suspension.
2. Through injection —in solution or fine suspension
—into bloodstream.

IX. *Exhibit*

Recording apparatus—

1. Of heartbeat rate.
2. Of respiratory rate.
   a. Observation by individual with microscope and stop-watch.
   b. Observation by several with microprojection and stop-watch.
   c. Observation by individual, microscopic observation recorded with Ingle apparatus.
   d. Observation by several, microprojection and photo-electric amplification and Kymograph recording.
   e. Observation by several, microprojection and photographic recording using metronome for time registration.
   f. Observation by motion picture.
3. Peristaltic movement—evacuation.
   a. Observation by individual and several. Microprojection of food canal onto ruled screen to provide uniform basis for judgment of degree and speed of evacuation.

X. *Standard reference substances*

1. Elaterin (preferably β fraction) for cathartic substances.
2. Yohimbine (tentative) for fixed depression of heartbeat.

XI. *Progress of research*

1. Increased staff and scope of Gross Research Laboratory.
2. New research grants: Merck and Company, Sandoz Chemical Works, Inc., etc.
3. Publications, reprints, and records of work completed.
4. Research under way:
   a. Studies of laxative substances.
   b. Studies of vitamin substances, especially vitamin E.
   c. Histo-cytological studies.
   Demonstration: Fixation through rapid dehydration without distortion (Dr. Cohen's method, using butyl alcohol saturated with water followed by ethyl alcohol (95%) butyl alcohol 100%—paraffin).
   d. Extremely delicate microphysiological, microtoxicological tests for active substances, poisons, narcotics, venoms, etc.

XII. *Collaboration work under way*

With Hahnemann School of Medicine, University of Illinois College of Medicine, Temple School of Pharmacy, etc.

It is suggested that the work be continued with collaborators.

REPORT ON HEXYLRESORCINOL

By M. L. YAKOWITZ (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

According to the Prescription Ingredient Survey of 1933,[1] the olive oil solution of hexylresorcinol is the most widely prescribed preparation containing hexylresorcinol. Work was therefore initiated on the determination of hexylresorcinol in this preparation.

Formerly commercial hexylresorcinol was described in New and Non-Official Remedies.[2] According to this source, commercial hexylresorcinol may contain up to 5 per cent of an intermediate compound, hexylylresorcinol. The sample of hexylresorcinol used in the writer's experiment was supplied by the Sharp and Dohme Company and contained an unknown amount of hexylylresorcinol.

Hexylresorcinol may be dried safely at 70° C., just above its melting point. It does not volatilize at all at this temperature during a period of two hours. It is not at all hygroscopic. Therefore, ether or chloroform solutions of hexylresorcinol may be evaporated at their boiling points and then dried at 70° C. to obtain a residue of pure hexylresorcinol.

Hexylresorcinol is an exceedingly weak acid. Thus it is not at all extracted from an ether or chloroform solution by 5 per cent sodium carbonate solution. However, it is completely extracted from such solvents by 2 per cent sodium hydroxide solution.

Unfortunately, hexylresorcinol is unstable in alkaline solution. Such solutions rapidly become colored and part of the hexylresorcinol is apparently oxidized to a compound more acidic in nature than the hexylresorcinol. This is shown by the following experiment: A weighed portion of hexylresorcinol was dissolved in 2 per cent sodium hydroxide solution and allowed to stand for 20 minutes. The solution was then made acid and the hexylresorcinol was extracted with chloroform. The chloroform solution was then extracted with one-fifth its volume of 5 per cent sodium carbonate solution, washed with water, and evaporated in a tared vessel. The weight of hexylresorcinol recovered amounted to but 92 per cent of the original weight of hexylresorcinol.

By acidifying the sodium carbonate solution and extracting with chloroform it was found that the sodium carbonate solution had extracted the altered hexylresorcinol. It was also found that the presence of hydrazine, a strong reducing compound, tended to prevent this alteration of hexylresorcinol in alkaline solution.

A method evolved for the determination of hexylresorcinol in olive oil solution rests upon the following principles. The sample of hexylresorcinol in olive oil is diluted with ether and extracted with a solution containing

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[1] Published by the American Pharmaceutical Association.
[2] 1933 Ed., published by American Medical Association.

barium hydroxide, sodium hydroxide, and hydrazine. The barium hydroxide precipitates the fatty acids, thereby preventing them from going into solution in the alkaline medium and also preventing the formation of a permanent emulsion. The hydrazine prevents oxidation of the hexylresorcinol. This alkaline extract is washed with ether and made acid, and the liberated hexylresorcinol is extracted by chloroform. The chloroform solution is washed with a sodium carbonate solution to remove any acidic compounds, and then washed with water. It is then evaporated, and the hexylresorcinol is dried to constant weight at 70° C.

HEXYLRESORCINOL IN OLIVE OIL

REAGENTS

(a) *Barium hydroxide*.—Prepare a saturated solution. Dilute with an equal volume of 2% NaOH solution. To 125 cc. of this mixture (volume required for one determination) add 1.5 cc. of 45% hydrazine hydrate solution

(b) *Ether*.—Wash first with 1/5 its volume of 2% NaOH solution and then with water.

(c) *Sodium carbonate solution*.—5 grams of Na_2CO_3 plus 2 grams of $N_2H_4 \cdot H_2SO_4$ in each 100 cc.

DETERMINATION

Transfer a weighed portion of sample containing about 0.25 gram of hexylresorcinol to a separator, add 50 cc. of ether, and extract successively with 50, 25, 25 and 25 cc. of solution A. Wash each portion of Solution A through two separators, each containing 25 cc. of ether. Add each portion of Solution A to a fourth separator containing 25 cc. of $CHCl_3$ and 10 cc. of concentrated HCl, shaking after the addition of each portion of Solution A. Shake out this acidified extract with four successive portions of 25 cc. of $CHCl_3$. Shake each extract successively through four separators containing in order, 10 cc of water, 10 cc. of the Na_2CO_3, another 10 cc. portion of Na_2CO_3, and 10 cc. of water. Filter the $CHCl_3$ from the last separator through a pledget of cotton into a tared evaporating dish. Evaporate the combined $CHCl_3$ extract on the steam bath until reduced to a volume of about 3 cc. Evaporate the last portion of $CHCl_3$ below the boiling point. Dry the residue at 70° C. during 15 minute intervals until constant weight is obtained. As a measure of purity the melting point of this residue is obtained It should be at least 65° C. Report hexylresorcinol in percentage of weight of sample taken.

When hexylresorcinol without olive oil was run through this method, recoveries of 97.5 and 98.5 per cent were obtained. When 10 gram portions of olive oil were run through the method, residues of 1–2 mg. were obtained.

A solution of hexylresorcinol in olive oil was made up to contain 1.486 per cent of hexylresorcinol by weight. A portion of this sample, assayed by the Associate Referee using the same method, yielded an amount of hexylresorcinol equal to 1.485 per cent of the sample. The residue obtained melted at 66° C.

P. S. Jorgensen of the San Francisco Station assayed this sample and obtained results of 1.481 per cent and 1.478 per cent of hexylresorcinol. The residue obtained by him melted at 65–66.5° C. H. W. Gerritz of the San Francisco Station also assayed this sample and obtained results of

1.479 and 1.461 per cent of hexylresorcinol. The residue melted at 65–66.5° C.

SUMMARY AND RECOMMENDATION

A method for the determination of hexylresorcinol in olive oil solution is presented. This method apparently gives good results when tried on a mixture containing a known amount of hexylresorcinol. It is recommended that the method be studied further in order to obtain more collaborative results and also to extend the method to other preparations containing hexylresorcinol. The fate of hexylresorcinol when carried through the method should also be determined.

Assay of hexylresorcinol in olive oil

COLLABORATOR	THEORY	FOUND	RECOVERY
	per cent	*per cent*	*per cent*
P. S. Jorgensen	1.486	1.481	99.66
		1.478	99.66
H. W. Gerritz	1.486	1.479	99.53
		1.461	98.32
M. L. Yakowitz	1.486	1.485	99.93

REPORT ON ERGOT ALKALOIDS

By C. K. Glycart (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

This subject was continued in accordance with the recommendations made last year.

After the discovery by Dudley and Moir[1] that ergot contained the potent water-soluble alkaloid ergometrine (1935), Hampshire and Page[2] were the first to investigate methods for the separate essay of ergometrine and ergotoxine (1936).

The preliminary work last year included a study of the Hampshire-Page method adapted to the assay of fluidextract of ergot, *This Journal*, **21**, 566 (1938). The Palkin-Murray-Watkins continuous extraction apparatus was used to remove the total alkaloids. It was found that the emulsions formed carried more pigment into the hot ether solvent in the receiving flask, consequently the separator procedure was studied this year. A patented product of liquid extract of ergot labeled "Biologically standardized... each cc. contains 0.1 mgm. of ergonovine" was sent to the collaborators.

[1] *Pharm J.*, **80**, 709 (1935).
[2] *Quart. J Pharm. Pharmacol.*, **9**, 60 (1936).

The method submitted was the one presented last year, except that the directions for the extraction of total alkaloids were changed by substituting the separators for the continuous extractor.

Summary of results

COLLABORATOR	TOTAL ALKALOIDS AS ERGOTOXINE	WATER-INSOLUBLE ALKALOIDS AS ERGOTOXINE	ERGOMETRINE	
	gram per 100 cc.	*gram per 100 cc.*	*gram per 100 cc.*	
C. H. Hampshire	(1) 0.035	0.015	0.0108	Klett
and G. R. Page	(2) 0.034	0.016	0.0097	
London, England				
W. I. Smith				
Public Health	0.036	0.018	0.0097	Klett
Washington, D. C.				
Chemical Control				
Laboratories	0.036	0.020	0.0086	Colorimeter
Eli Lilly & Co.	0.035	0.024	0.0059	
Indianapolis, Ind.				
F. C. Ferger	0.038	0.017	0.0113	Klett daylight
Abbott Labs.	0.032	0.014	0 0097	Artificial light
North Chicago, Ill.				
A. Taub	0.036	0.021	0.0081	Spectro-
Columbia University	0.033	0.020	0.0070	photometer
New York City				
Lloyd C. Miller	0.0334	0.0183	0.0081	Spectro-
U. S. Food & Drug. Adm.	0.0359*	0.0196*	0.0088*	photometer
Washington, D. C.				
C. K. Glycart	0.038	0.020	0 0097	Klett
U. S. Food & Drug Adm.	0.035	0.018	0.0091	
Chicago, Ill.				

* Values obtained when the color intensity was read at a wave length of 550 mμ and compared with the corresponding data obtained on the standard ergotoxine solution supplied. Since submitting my report, additional determinations of the color intensity at various wave lengths have been made with pure ergotoxine and ergonovine. I feel that 550 mμ is probably a better wave length at which to measure the color than is 660 mμ.

COMMENTS

*C. H. Hampshire.**—Our principal comment on your paper is that you do not produce any evidence, based upon analyses of extracts or solutions of known strength, to show that the methods you use extract all the alkaloid from the aqueous medium.

We have found that by the use of the Watkins apparatus it is possible to effect practically complete extraction of the ergometrine from aqueous solution by ether.

* This collaborator is Secretary of the British Pharmacopoeia Commission He stated in his comments that ergometrine is incompletely extracted with ether by the separator procedure since Hampshire and Page determined that the partition coefficient of ergometrine for ether is about 8.1 in favor of water, and that therefore after shaking out the alkaloids with 40, 20, 20, 10, and 5 ml. of ether, only 65% will be extracted in this way.

When we applied this method to the liquid extracts of ergot of the British Pharmacopoeia, . . . we found that the ether extracted much colouring matter. On shaking the ether with tartaric acid solution the colour passed into the aqueous layer and rendered this too dark for use in the colorimetric determination . . .

We would make a further comment on the proposed method of assay in regard to the test for complete extraction by ether. The amount of alkaloid removed from a small quantity of the ethereal extract gives a very faint colour and it would be quite easy for a worker to assume that complete extraction had been reached long before such was the case. In the later stages of the shaking out process, it is necessary to use a large quantity of the ether extract to get a measurable colour and, should appreciable amounts of ergometrine be present in this, the result of the final assay will be, of course, too low . . .

Abraham Taub.—A Bausch and Lomb spectrophotometer was used. Results were computed from curves obtained with standard solutions containing 0.2, 0.4, 0.6, 0.8, and 1.0 cc. of standard solution. Comparisons were made at 550 mμ. Identical readings were obtained at 600 mμ. As the curve between 550 and 600 is flat, it should be possible to obtain precise results with an ordinary colorimeter and a filter having a transmission limited to 5500 to 6000 A.U. I found no difficulty in measuring the ether solutions at 20° C.

F. C. Ferger.—Used five 20 cc. portions of water to extract water-soluble alkaloids. Hard to extract—alkaloids seem to be about as soluble in ether as in NH_4OH (1+1). An amount of standard solution should be used to more nearly approximate the color produced in the unknown.

W. J. Rice, Eli Lilly & Co.—In the absence of the photometer our work has been confined to the colorimetric determinations. It will be noted that two complete assays were made and that while the results check very well for total alkaloids they do not agree in the determination of water-insoluble alkaloids nor in the determination of ergometrine. In the absence of sufficient material to further verify these results, we do not feel prepared to draw any definite conclusions on the basis of this work.

Lloyd C. Miller.— . . . The color determinations were made by means of a spectrophotometer, which is well adapted to such color comparisons. Readings were taken at 660 mμ. According to Clifford, *This Journal*, 19, 130 (1936) this wave length corresponds to that given by the number 66 filter prescribed in the text of the method supplied. We have found that the same results are not obtained when other wave lengths than 660 mμ are used, but as yet we are not in a position to recommend another wave length as being better.

Regarding the method of dividing the ether, the procedure specified in the directions is probably as good as any that is practical and simple. Two suggestions may be made for other means of accomplishing the division of the extract into two aliquots. Calvery has suggested (private communication) that a 100 cc. graduated separator might be used. This appears particularly promising, since the first 50 cc. aliquot may be removed and the second may be extracted with water, directly, without removal from the separatory funnel. A second suggestion, which occurred to the writer, is that a "sugar" volumetric flask might be used. These flasks have graduations at 100 and 110 cc. and are obtainable at any chemical supply house. In using them, two aliquots equivalent to 5/11ths could then be taken in identical fashion.

SUMMARY

The separator procedure studied this year is inadequate for the extraction of the total alkaloids of ergot since the partition coefficient of ergometrine in ether and water is about 8 to 1 in favor of water, as

shown by Hampshire in his comments. With the continuous extractor the alkaloids were removed completely in 5 hours. This process also removes considerable pigment matter, which interferes with the colorimetric reading.

Ferger reported that colorimetric results obtained by artificial light differ from those obtained by daylight. It is believed that these difficulties may be overcome by the use of the Clifford wedge photometer and suitable filters.

The problem is considered to be still in the preliminary stages of study. It appears that there is an urgent need for a separate assay method of ergometrine and ergotoxine.

It is recommended[1] that methods for ergot be further studied.

REPORT ON NITROGLYCERINE IN MIXTURES

By OMER C. KENWORTHY (U. S. Food and Drug Administration, New York), *Associate Referee*

Last year's report to the Association, *This Journal*, **20**, 569 (1937) showed that the acid distillation method was workable for most mixtures, but the presence of iron, caffeine, and various plant extracts in a mixture gave high results, approximately 50 per cent more nitroglycerine than was present.

Preliminary experiments this year to determine the reason for this discrepancy indicated that it was the iron and plant extracts in combination that gave the high results. Iron and caffeine offered no interference, the yield being practically 100 per cent. As iron and plant extracts are common ingredients in complex nitroglycerine tablets, the method must work in their presence.

In the belief that perhaps some acid other than sulfuric might be preferable as an acidifying agent in the acid distillation, various other acids were tried. Of these, phosphoric, hydrochloric, and acetic gave high results (from 20 to 50 per cent excess) while boric, citric, and tartaric gave low results. In fact, the citric and tartaric acids in the amounts used (5 grams) destroyed all the nitroglycerine. The pH of these solutions was not taken, but one determination using 50 cc. of a buffer solution of disodium phosphate and monosodium phosphate in equal amounts (which had a pH of 5.6) gave a result about 25 per cent in excess.

In view of the varying results obtained, the Associate Referee decided to drop any attempt at a solution of the problem from this angle and to separate the iron from the mixture by means of the A.O.A.C. alcohol aliquot method before making the acid distillation, *Methods of Analysis, A.O.A.C.*, 1935, 563, **60**.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 65 (1938).

To a 250 cc. glass-stoppered Erlenmeyer flask were added 2 grams each of powdered extract of belladonna leaves, hyoscyamus, nux vomica, and digitalis; 2 grams each of caffeine and reduced iron; 4 grams of calcium carbonate; and then the nitroglycerine, which was in the form of powdered hypodermic tablets. Exactly 50 cc. of alcohol containing 2 cc. of 10 per cent sulfuric acid was added to the mass, and the flask was stoppered and shaken for 5–10 minutes. The mixture was allowed to settle, then decanted through a dry filter paper; 25 cc. of the filtrate was decanted into an 800 cc. Kjeldahl flask, and the acid distillation was made.

This method gave a recovery of nitroglycerine of 96–99 per cent on four determinations. It is of interest here to note that two samples, where the nitroglycerine was allowed to remain in contact with the dry ingredients for approximately two weeks before the assay was completed, gave a recovery of nitroglycerine of only 80 and 82 per cent. The method was tried with both plain alcohol and acidulated alcohol as the solvent and both appeared to work equally well. However, it seemed to be safer to use the acidulated alcohol as a general method.

Samples were sent to collaborators to be tested by the method outlined below:

NITROGLYCERINE IN MIXTURES

REAGENTS

(a) *Sodium sulfate.*—Saturated solution.

(b) *Acidulated alcohol.*—To 50 cc. of alcohol add 2 cc. of 10% H_2SO_4

DETERMINATION

Determine the average weight of the tablets.

Powder 40 tablets in a mortar, weigh the powder, and transfer completely to a 125 cc. glass-stoppered Erlenmeyer flask. Add exactly 50 cc. of the acidulated alcohol, stopper the flask, and shake for 5–10 minutes. Allow the mixture to stand 15–20 minutes and decant through a fluted filter paper. Pipet 25 cc. of the filtrate into an 800 cc. Kjeldahl flask containing 2 cc. of 10% H_2SO_4 and 2 or 3 glass beads. Add 50 cc. of the Na_2SO_4 solution and 150 cc. of water. Distil just to dryness, using a distilling trap, and collect the distillate in an 800 cc. Kjeldahl flask containing 30 cc. of a 5% NaOH solution. Use a moderate flame to prevent any non-volatile matter being carried over by the spray. Disconnect and wash the condenser and outlet tube with about 100 cc. of water, receiving the washings in the flask containing the alkali. Add 2 grams of Devarda alloy and distil the ammonia into 25 cc. of 0.02 N H_2SO_4, using a spray-type distilling flask. Titrate the excess acid with 0.02 N NaOH, using methyl red indicator. Use care as there is considerable foaming during the early part of the distillation.

1 cc. 0.02 N acid = 0.001514 gram of nitroglycerine.

(a) Run 1 determination on the tablets alone by the above method.

(b) Run 1 determination on the tablets by weighing out 20 tablets and adding them directly to the Kjeldahl flask beginning with "800 cc. Kjeldahl flask containing 2 cc. of 10% H_2SO_4."

(c) Run 2 determinations by the above method, using in addition to the 40 tablets 16 grams of the "mixture," to be added to the Erlenmeyer flask.

The nitroglycerine was in the form of hypodermic tablets. The so-called mixture contained in each 16 grams, 2 grams each of powdered extracts of digitalis, hyoscyamus, belladonna leaves, and nux vomica; 2 grams each of reduced iron and caffeine; and 4 grams of calcium carbonate.

The results of the collaborators, expressed as grains per average tablet, are given in the table.

	NITROGLYCERINE (GRAMS/TABLET)		
	ON WHOLE TABLETS	ON POWDERED TABLETS	ON POWDERED TABLETS +16 GRAMS OF MIXTURE
C. F. Bruening	0.0270	0.0264	0.0196
F. & D. Administration		0.0266	0.0217
Baltimore, Md.			0.0204
			0.0194
F. A. Rotondaro	0.0265	0.0273	0.0226
F. & D. Administration	0.0268	0.0270	0.0222
Philadelphia, Pa.			0.0215
O. C. Kenworthy	0.0265	0.0255	0.0187
F. & D. Administration	0.0265	0.0257	0.0188
New York City	0.0263	0.0268	0 0204
	0.0260		
	0.0263		

CONCLUSIONS

Although the method seems promising, it is not as yet satisfactory for mixtures containing nitroglycerine in the presence of reduced iron and plant extracts.

It has been shown, however, that the tablets may be powdered before being assayed without any significant loss of nitroglycerine.

It is recommended[1] that the study be continued.

REPORT ON GUAIACOL

By K. L. MILSTEAD (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Guaiacol is not a new subject so far as the Association of Official Agricultural Chemists is concerned. An associate referee was first appointed in 1929, and reports were published in 1931, 1932, and 1933. A second associate referee was appointed in 1933 and it was recommended by him that no further study be made on this particular phenol. However,

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 65 (1938).

the Association appointed a third associate referee in 1935. No report was given by him.

The U. S. Pharmacopoeia recognizes guaiacol as "a liquid consisting principally of $C_6H_4(OH)(OCH_3)$ 1:2, usually obtained from wood creosote, or a solid, consisting almost entirely of $C_6H_4(OH)(OCH_3)$ 1:2, usually prepared synthetically." Physical and chemical tests for purity are given for both the liquid and solid guaiacol, but there is no method of assay. It is obvious that the liquid guaiacol consists of a mixture of guaiacol and closely related phenols, known as creosols, in variable proportions, and that it would be desirable to develop a method that would establish the true guaiacol content of such a mixture.

The method reported in this paper does not distinguish guaiacol from creosols, but it does offer methods for, (a) the determination of pure guaiacol, (b) the determination of wood creosote (by establishing limits for the "guaiacol like compounds" as determined by this method in authentic samples of wood creosote), and (c) the determination of guaiacol derivatives.

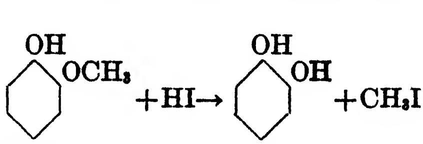
REVIEW OF THE LITERATURE

The first associate referee directed his efforts toward effecting a determination of guaiacol by the application of methods used for the determination of related compounds. He reviewed the literature, *This Journal*, **14**, 367 (1931); **15**, 429 (1932); **16**, 369 (1933), and applied methods that seemed feasible. The colorimetric method with Folin-Denis reagent, the gravimetric bromination method applied by Chernoff to guaiacol carbonate, the sodium bromate titration method applied to the estimation of thymol by Hart, the bromate-thiosulfate method and acetylation under pressure were all studied, but none of them yielded consistent results.

Very few other attempts have been made in this direction, although a method reported by Francois and Sequin[1] should be noted. These investigators found that when guaiacol was treated with iodine and potassium iodide under carefully controlled conditions, the iodine compound obtained represented a recovery of 99.78 per cent of the original guaiacol. Although this method was not studied by the present Associate Referee, it suggests a future course of investigation.

BASIS OF PRESENT METHOD AND APPARATUS USED

The method developed depends on the fact that guaiacol contains a methoxy group which when treated with hydriodic acid undergoes the following reaction:



[1] *Bull. Soc. Chem.*, **53**, 711–23 (1933).

The methyl iodide is determined by the Viebock and Schwappach[1] method as modified by E. P. Clark, *This Journal*, **15**, 136 (1932). The methyl iodide is collected in an acetic acid solution of potassium acetate to which a little bromine is added. The following reactions then occur:

$$CH_3I+Br_2 \rightarrow CH_3Br+IBr$$
$$IBr+2Br_2+3H_2O \rightarrow HIO_3+5HBr$$

The solution containing the iodic acid is washed into a flask containing a little sodium acetate, the excess bromine is removed with formic acid, potassium iodide is added, the solution is acidified with sulfuric acid, and the liberated iodine is titrated with 0.1 *N* thiosulfate.

It is obvious that the method is not specific for guaiacol but is rather an estimation of alkoxyl groups, and that any compound containing such a group would interfere with the determination. It is, however, more specific than any estimation that would depend on the phenolic properties of the compound. The method has the further advantage that the guaiacol can be determined whether free or combined in the form of many of its compounds.

Only one modification has been introduced in Clark's procedure. It was found that hydriodic acid that yielded a low and constant blank by treatment at 100° C. with hypophosphorous acid could not be obtained. In order to obtain a satisfactory blank it was necessary to boil the hydriodic acid with considerable excess of hypophosphorous acid under a reflux condenser for 30 minutes.

EXPERIMENTS WITH GUAIACOL CARBONATE

The precision of the method was first tested with guaiacol carbonate since this compound can be obtained in almost pure form and is easily handled.

Portions of guaiacol carbonate, conforming to N.F. VI purity tests and dried to constant weight over sulfuric acid, were weighed on the analytical balance, and the determination was completed as described under the method.

In Table 1 is recorded the results obtained by the Associate Referee and one collaborator.

The results (Table 1) indicate that guaiacol carbonate can be determined by this method with a precision of 0.3 parts per thousand.

EXPERIMENTS WITH GUAIACOL

Synthetic guaiacol that conformed to the U.S.P. XI purity tests was used in this investigation.

The standard guaiacol solution was prepared by adding about one gram of guaiacol to a small, accurately weighed glass-stoppered bottle containing 5 cc. of 25 per cent sodium hydroxide and weighed again after

[1] Hans Meyer, Analyse und Konstitutions Ermittlung, 5 auf, p. 487.

TABLE 1.—*Results obtained by Associate Referee and one collaborator*

TRIAL		TRIAL SAMPLE TAKEN	GUAIACOL CARBONATE FOUND	RECOVERY	DEVIATION FROM ARITHMETICAL MEAN
		mg.	*mg.*	*per cent*	*per cent*
Associate	(1)	59.4	59.3	99.83	+0.01
Referee	(2)	73.6	73.5	99.86	+0.04
	(3)	82.9	82.7	99.76	−0.06
Maurice	(1)	50.5	50.4	99.80	−0.02
Harris	(2)	71.2	71.1	99.85	+0.03
	(3)	98.1	97.9	99.80	−0.02
				Average deviation	0.03
Arithmetical mean				99.82	
Average deviation/arithmetical mean				0.3 parts/1000	

reaching room temperature. The contents of the bottle were washed into a 100 cc. volumetric flask and made to the mark with water at 20° C. All aliquots for analysis were removed at 20° C.

Accurately measured aliquots of different alkaline guaiacol solutions prepared as above were placed in the boiling flask and evaporated just to dryness on the steam bath in a current of air. The flask was then removed and connected with the remainder of the apparatus, and the determination was completed as described in the method.

Results obtained with varying amounts of guaiacol and under varying conditions are recorded in Table 2.

TABLE 2.—*Results obtained under varying conditions*

TRIAL	TRIAL SAMPLE TAKEN	AGE OF ALKALINE GUAIACOL SOLUTION	METHOD OF TREATMENT OF ALKALINE GUAIACOL SOLUTION	GUAIACOL FOUND	CALCULATED PURITY
	mg.	*days*		*mg*	*per cent*
1	52.5	0	Evaporated just to dryness	52.0	99.04
2	52.5	9	Evaporated just to dryness	51.9	98.85
3	21.0	10	Evaporated just to dryness	20.8	99.04
4	42.0	11	Heated 5 minutes after reaching dryness on steam bath	41.1	97.85
5	46.9	1	Heated 5 minutes after reaching dryness on steam bath	46.1	98.29
6	65.7	2	Evaporated just to dryness	64.6	98.33
7	21.0	1	2 cc. aliquot not evaporated	17.3	82.38
8	46.9	4	Evaporated on steam bath and heated 1 hour after reaching dryness	45.4	97.81

Summary of Table 2

Number of determinations..........	5
Range..........................	98.29–99.04%
Average........................	98.71%

The precision obtained in the five determinations by the Associate Referee following the method is three parts per thousand.

The loss of 1.29 per cent of the theoretical guaiacol may be accounted for partly by the fact that pure guaiacol is very difficult to obtain. The product used in this investigation was re-distilled, and the fraction boiling between 204.5 and 205.0° C. was used. It may have contained a small amount of other compounds. Recent work with this method on a related product indicates that the sodium hydroxide may cause a lower recovery, although no work has been done on guaiacol in this direction.

Alkaline guaiacol solutions may be allowed to stand 10 days without affecting the recovery. (Trials 1, 2, and 3 were made on the same solution.) On longer standing the recovery may decrease (Trial 4). Prolonged heating after dryness has been reached may give a slightly lower recovery (Trial 8). The evaporation of the solution to dryness in order to eliminate most of the water is necessary (Trial 7).

EXPERIMENTS WITH U S.P. LIQUID GUAIACOL

In order to determine the "guaiacol-like compounds" in liquid guaiacol, an alkaline solution of U.S.P. liquid guaiacol was prepared as directed under "Experiments With Guaiacol."

Aliquots of the alkaline solution were analyzed according to the proposed method and the results shown in Table 3 were obtained.

TABLE 3.—*Results obtained by the proposed methods*

TRIAL	TRIAL SAMPLE TAKEN	GUAIACOL-LIKE COMPOUND FOUND	RECOVERY CALCULATED AS GUAIACOL
	mg.	*mg*	*per cent*
1	52 0	41.2	79.23
2	52 0	41.5	79 80

The results (Table 3) indicate that U.S.P. liquid guaiacol contains about 80 per cent of "guaiacol-like compounds" calculated as guaiacol.

EXPERIMENTS WITH POTASSIUM GUAIACOL SULFONATE

Two samples of potassium guaiacol sulfonate conforming to N.F. VI purity tests were analyzed by the procedure outlined for guaiacol carbonate. Sample A was dried over sulfuric acid for six days, and the determination was made on the dry salt. Sample B was analyzed by Harris after it had been dried over sulfuric acid for six days and by the Associate Referee without drying. The moisture content of Sample B was determined by the Associate Referee, and the results were corrected to the anhydrous basis (Table 4).

TABLE 4.—*Moisture results on Samples A and B by proposed method*

SAMPLE	TRIAL	ANALYST	TRIAL SAMPLE TAKEN	POTASSIUM GUAIACOL SULFONATE RECOVERED	CALCULATED PURITY
			mg.	*mg.*	*per cent*
A	1	Associate Referee	44.5	40.7	91.46
	2	Associate Referee	78.3	72.2	92.20
B	1	Associate Referee	50.6	48.6	96.04
	2	Associate Referee	102.9	98.3	95.54
	1	M. Harris	70.5	68.0	96.4

The results on two samples of potassium guaiacol sulfonate indicate that the commercial salt conforming to the N.F. VI purity tests is not entirely the pure compound. This is in agreement with the findings of other investigators,[1] namely, that commercial samples of potassium guaiacol sulfonate contain basic salt as well as disulfonated compounds.

EXPERIMENTS WITH WOOD CREOSOTE

An alkaline solution of beechwood creosote was prepared according to the method used for guaiacol. Aliquots of this solution were also analyzed according to the method.

An aliquot of the alkaline solution was added to 10 grams of brown sugar, and the solution was acidified and extracted with ether. The ether layer was evaporated and extracted with 1 per cent sodium hydroxide. An aliquot of the alkaline solution was then analyzed. The results obtained are shown in Table 5.

TABLE 5.—*Results on beechwood creosote*

TRIAL	TRIAL SAMPLE TAKEN	GUAIACOL-LIKE COMPOUND FOUND	RECOVERY CALCULATED AS GUAIACOL
	mg.	*mg.*	*per cent*
1	5.27	3.07	58.25
2	5.27	3.07	58.25

CREOSOTE ADDED TO 10 GRAMS SUGAR	CREOSOTE IN ALIQUOT FOR ANALYSIS	GUAIACOL-LIKE COMPOUNDS FOUND	RECOVERY
mg.	*mg.*	*mg.*	*per cent*
52.7	5.27	2.85	54.07
52.7	5.27	2.87	54.45

The analysis of a sample of beechwood creosote indicates that it contains about 58 per cent "guaiacol-like compounds" calculated as guaiacol.

[1] Rising, A. *Ber.*, 39, 3685 (1906).

There was a loss of about 4 per cent in the recovery of the creosote when added to brown sugar.

METHOD

REAGENTS

(a) *Phenol.*—Highest quality crystalline phenol.

(b) *Hydriodic acid.*—Sp. gr. 1.7. Boil the HI under a reflux condenser with an excess of hypophosphorous acid for 30 minutes. When cool, transfer to a dark glass-stoppered bottle. Do not allow the acid to stand with the stopper removed for more than a few minutes.

(c) *Glacial acetic acid—potassium acetate solution.*—10%. To 100 cc. of glacial acetic acid add 10 grams of C.P. potassium acetate.

(d) *Sodium acetate.*—25%. To 100 cc. of distilled water add 25 grams of C.P. sodium acetate.

APPARATUS

(1) *Boiling rod.*—Glass tube 60 mm. long, 3.5 mm. outside diameter, with a 1 mm. bore. It is sealed at one end and also closed about 10 mm. from the other. The rod is placed in the flask with the open end down.

(2) *Carbon dioxide.*—Cylinder of carbon dioxide.

(3) *Methoxy apparatus.*

DETERMINATION

Introduce an aliquot of the alkaline guaiacol solution (guaiacol dissolved in 1% NaOH) containing 30.0–60.0 mg. of guaiacol into the boiling flask and evaporate the solution just to dryness on the steam bath in a current of air.* For solid guaiacol compounds weigh accurately on a cigarette paper 60–100 mg. and introduce directly into the flask. Add 2.5 cc. of analytical quality phenol, 5 cc. of hydriodic acid, and a boiling rod. Connect the flask with the remainder of the apparatus, which consists of the scrubber, containing a little water, and the receivers. The receivers contain 10 cc. of glacial acetic acid-potassium acetate solution to which 10 drops of Br have been added. Approximately 6 cc. of the solution is placed in one and 4 cc. in the other.

Pass a slow uniform stream of CO_2 (about one bubble per second) through the capillary side arm of the boiling flask and heat the liquid gently with a micro burner at such a rate that the vapor of the boiling liquid rises half way up the condenser; after 30 minutes discontinue the heating but continue to pass CO_2 through the apparatus for a few minutes in order to carry over completely any methyl iodide.

Wash the contents of the receivers into a 250 cc. Erlenmeyer flask containing 5 cc. of sodium acetate solution. Adjust the volume of the liquid to approximately 125 cc. and add 8 drops of 90 per cent formic acid. Rotate the flask until the color due to the bromine is discharged, add 12 more drops of formic acid, and allow the flask to stand from 1 to 2 minutes. Add 5 cc. of 10% H_2SO_4, 1 gram of KI and titrate the liberated iodine with 0.1 N $Na_2S_2O_3$. Correct for the number of cc. required to titrate the blank run in the same way, using the same quantity of reagents.

1 cc. 0.1 N $Na_2S_2O_3 = 2.068$ mg. guaiacol;

2.28 mg. guaiacol carbonate; 4.036 mg. potassium guaiacol sulfonate.

* For solid compounds that can be weighed the same phenol and hydriodic acid will serve for many determinations. The contents of the flask A are allowed to cool, the sample introduced, and the determination continued.

GENERAL SUMMARY AND CONCLUSION

The Viebock and Schwappach method, as modified by E. P. Clark for the estimation of alkoxyl groups, has been applied to guaiacol, guaiacol carbonate, potassium guaiacol sulfonate, U.S.P. liquid guaiacol, and wood creosote.

The precision of the method applied to guaiacol carbonate is 0.3 parts per thousand and to guaiacol 3.0 parts per thousand.

The average recovery of six determinations on guaiacol carbonate was 99.82 per cent, and on five determinations of guaiacol it was 98.71 per cent.

U.S.P. liquid guaiacol contains about 80 per cent of "guaiacol-like compounds" calculated as guaiacol.

Potassium guaiacol sulfonate consists of more than one compound.

A sample of beechwood creosote contained about 58 per cent "guaiacol-like compounds" calculated as guaiacol.

The method is simple and practical and yields very accurate results with guaiacol carbonate and reasonably accurate results for guaiacol.

It is recommended[1] that the quantitative determination of guaiacol in wood creosote be studied and that the method as applied to guaiacol carbonate be submitted to collaborative study.

No report on biological testing was given by the associate referee.

REPORT ON IODINE OINTMENT

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Associate Referee*

As recommended last year, *This Journal*, **20**, 56 (1937), the methods proposed by the Associate Referee for the estimation of free iodine and organically combined iodine were subjected to collaborative study. The results obtained appear in the table.

Several collaborators, who wished to know how the 0.1 *N* potassium arsenite solution used in this laboratory was prepared, were referred to the method published in "Representative Procedures in Quantitative Analysis" by Frank Austin Gooch.

DISCUSSION AND COMMENTS OF COLLABORATORS

Little or no difficulty was experienced with the method for the determination of free iodine. One analyst stated that it was easier to observe the end point if a larger volume of water than 30 cc. was used, while another suggested weighing the sample in glass tubing and then dropping it into the iodine flask.

Several collaborators found the number of washings to be insufficient to remove all the iodine and iodide.

Agreement among the collaborators on the free iodine method is believed to be as satisfactory as can be expected, considering the unstable

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 66 (1938).

Collaborative results on iodine ointment

COLLABORATOR	FREE IODINE	ORGANICALLY COMBINED IODINE
	per cent	*per cent*
L. T. Ryan	3.70	0.38
	3.65	0.31
W. D. Dembeck	3.51	0.456
	3.49	0.453
	3.51	
	3.52	
J. Claggett Jones	3 31	0.674
	3 38	0 594
	3.32	0 402
		0.474
H. J. Fisher	3 38	1.15
	3.38	1 02
		1 16
H. E. Chaney	3 59	0 164
	3 35	0 150
	3.43	0 137
Associate Referee	3.30	0 462
	3.34	0 469
	3.33	0.451
E. H. Berry*	3.16	1.48
	3 15	1 24
Average	3.44	0.52

* These results were obtained after the material had been exposed to high summer temperatures and are not included in the averages

character of the product and the difficulty of obtaining uniform samples. Certainly there is good agreement among the individual results of each worker. The standard deviation from the mean value of 3.44 per cent, and within which two-thirds of the results should fall, is 0.12 per cent; 71 per cent of the determinations lie within these limits.

Collaborative work on the organically combined iodine method yielded disappointing results. Whether or not this is due to the difficulty of obtaining respresentative samples when a small amount of iodine is to be determined is hard to say.

The following comments and suggestions were made on the method for organically combined iodine.

L. T. Ryan.—The effectiveness of extraction was increased by adding about 5 cc. of cold water after each aqueous layer was poured off, washing with a rotatory motion, and pouring off this aqueous layer.

J. Claggett Jones.—The last two samples were run without being transferred to a crucible, the potassium carbonate was added, and the method followed from there on, the heating being done, as directed, in the same beaker. The beaker was not damaged, the transfer difficulty was eliminated, and no spattering was encountered.

RECOMMENDATIONS[1]

It is recommended—

(1) That the proposed method for the determination of iodine in iodine ointment be adopted as tentative. The method was published in *This Journal*, **21**, 94 (1938).

(2) That the method for the determination of organically combined iodine in iodine ointment be further studied.

No final report on acetophenetidin in presence of caffeine and aspirin was given by the associate referee.

REPORT ON PYRIDIUM

By Harry J. Fisher (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

Last year a method for the determination of pyridium by titration with titanous chloride was partially worked out, *This Journal*, **20**, 576 (1937). Due to the fact that the method was not then wholly satisfactory as applied to pyridium ointment, the details of the method were not given in the report.

This year the Associate Referee continued his study of the application of the method to ointments. For some time he was unable to obtain recoveries of more than 92 per cent. The difficulty was thought to lie in incomplete extraction of pyridium from the ether solution of the ointment. Extraction appeared particularly difficult when the ointment was prepared with pyridium base instead of pyridium itself (the hydrochloride). On the theory that combination might have taken place between the pyridium base and the fatty acids of the ointment, the experiment was tried of reflexing the ointment with concentrated hydrochloric acid before dissolving in ether and extracting with hydrochloric acid and water. Recoveries fell to 57 per cent. The ether solution of the ointment was highly colored after prolonged extraction with acid; the acid washings were yellow instead of pink; the color remaining in the ether was immediately removed on shaking the ether solution with dilute ammonia. It was evident that the refluxing with hydrochloric acid had decomposed the dye.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 66 (1938).

The true cause of the low results was therefore a partial decomposition of pyridium by strong hydrochloric acid during the evaporation of the acid washings on the steam bath. To avoid this decomposition, instead of evaporating the combined acid washings they were made ammoniacal and extracted with chloroform, and the chloroform extract was evaporated. When the residue was dissolved in dilute hydrochloric acid and titrated with titanous chloride in the usual manner, theoretical results were obtained.

The method as finally adopted was published in *This Journal*, **21**, 94 (1938).

EXPERIMENTAL RESULTS

Pure pyridium base was prepared by dissolving commercial pyridium in hot water, precipitating with ammonia, cooling, filtering, recrystallizing from hot water, and drying at 100° C.

When 0.1 gram portions of this were dissolved in 10 cc. of 0.1*N* hydrochloric acid, diluted to 100 cc., and titrated according to the method, three determinations gave 99.53, 100.1, and 99.73 per cent of pyridium base, an average of 99.79 per cent.

The theoretical percentage of pyridium base in pyridium ($C_{11}H_{11}N_5 \cdot HCl$) is 85.39. Two titrations of a sample of pyridium showed 85.77 and 85.02 per cent of pyridium base, an average of 85.40 per cent.

A commercial sample of 0.1 gram pyridium tablets when analyzed by the method showed 0.1044 and 0.1070 gram of pyridium per tablet.

Two determinations on a commercial sample of 1 per cent pyridium jelly gave 0.995 and 1.000 per cent of pyridium.

COLLABORATIVE STUDY

Due to the amount of time consumed in working out the method, it was not possible to submit it to collaborative study by other laboratories. Two samples were analyzed by two other chemists in this laboratory, however. Sample No. 6127 was an aqueous solution containing 1 gram of pyridium and 10 grams of dextrose in 100 cc.; No. 6128 was a 10 per cent pyridium ointment prepared according to the formula given in New and Nonofficial Remedies.[1] Averages of several determinations were as follows:

Per cent pyridium found

COLLABORATOR	SAMPLE NO. 6127	SAMPLE NO. 6128
Mathis	0.99	10.24
Shepard	1.01	10.30
Fisher	0.96	10.17

[1] New and Nonofficial Remedies, 1932, p. 159.

It is recommended[1] that the proposed method for pyridium be studied collaboratively during the coming year.

The paper on volatile oil in cinnamon listed on the program was not presented.

The paper on lead oleate plaster listed on the program was not presented.

No report on gums was given by the associate referee.

REPORT ON CINCHOPHEN IN PRESENCE OF SALICYLATES

By ALBERT I. COHEN (Armour Institute of Technology, Chicago, Ill.), *Associate Referee*

In compliance with the recommendations of Subcommittee B, a method for cinchophen in the presence of salicylates was developed and submitted to collaborative study.

Rabak, *This Journal*, **7**, 33 (1923), mentions that cinchophen forms an addition compound with iodine. Stainier and Massart[2] determine cinchophen in the presence of salicyclic acid by precipitating the former as the iodine addition compound, liberating the cinchophen with thiosulfate solution, and weighing it directly. Emery[3] estimates cinchophen by adding an excess of standardized iodine solution and determining the quantity of reagent consumed by back-titration with standard thiosulfate solution. The procedure recommended in this report is derived from the Emery method. The cinchophen is precipitated as the addition compound, $(C_{16}H_{11}NO_2)_2 \cdot HI \cdot I_3$, and its quantity is calculated from the concentration of iodine in an aliquot portion of the filtrate. The method was published in *This Journal*, **21**, 95 (1938).

The collaborative samples consisted of a powdered mixture containing 33.3 per cent cinchophen, 33.3 per cent aspirin, 11.2 per cent starch, 11.1 per cent talc, and 11.1 per cent stearic acid. The analytical findings are given in the table. They are in excellent agreement.

It is recommended[4] that the method presented be adopted as tentative and that the subject of cinchophen in the presence of salicylates be closed.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 66 (1938).
[2] *Quart J Pharm Pharmacol.*, **9**, 304 (1936).
[3] *J. Am Pharm. Assoc.*, **17**, 18 (1928).
[4] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 66 (1938).

Collaborative results

COLLABORATOR	WEIGHT OF SAMPLE	CINCHOPHEN FOUND	RECOVERY
	gram	*per cent*	*per cent*
Irwin S. Shupe	0.491	33.4	100.3
St. Louis	.459	33 1	99.4
S. Reznek	.500	33.1	99 4
New York	500	33 0	99.1
Albert I. Cohen	.441	33.0	99 1
	.495	33.4	100.3

REPORT ON THEOBROMINE IN THEOBROMINE-CALCIUM TABLETS

By P. S. JORGENSEN (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

Theobromine is a basic principle found in the fruit of the cocoa plant (*Theobroma cacao*), in the leaves of the teaplant (*Thea sinensis*), and in the Kola nut (*Cola acuminata*). Chemically it is 3, 7-dimethylxanthine, isomeric with theophylline, but differs in the location of the methyl groups, which in theophylline are in the 1, 3 positions.

Theobromine is relatively insoluble in the usual solvents including water, but its solubility in water is increased considerably by combining it with salts. It is as these double salt combinations that it is used in medicine. The product discussed in this investigation is theocalcin, a double salt or mixture of theobromine calcium and calcium salicylate.[1]

The periodide method of Emery and Spencer[2] was applied by E. O. Eaton, *This Journal*, **19**, 534 (1936), for the quantitative estimation of theobromine in theobromine calcium with apparently satisfactory results. The method proposed in this report is accurate and much less time consuming. It is described by H. Boie[3] for the acidimetric estimation of theobromine in combination with various salts. It is based on the fact that silver nitrate reacts with theobromine to form insoluble silver theobromine and liberating nitric acid according to the following equation:

$$C_7H_8N_4O_2+AgNO_3 \rightarrow AgC_7H_7N_4O_2+HNO_3.$$

The sample is put in solution, the acidity is adjusted, silver nitrate is added, and the liberated nitric acid is titrated with 0.1 *N* sodium hydroxide. The method with a few modifications is applicable to theobromine

[1] New and Non-official Remedies, 1936, p 466
[2] *J. Ind. Eng. Chem*, **10**, 605 (1918).
[3] *Pharm. Ztg.*, **75**, 968 (1930); *C A.*, **25**, 169 (1931).

alkaloid, to the salt of theobromine with calcium salicylate (theocalcin), and to this salt in combination with excipients ordinarily used in tablets. Experiments indicate that it is applicable to theophylline and its salts also.

Samples of the powdered theocalcin tablets, theocalcin powder, and pure theobromine alkaloid were sent to several collaborators with the following directions:

INSTRUCTIONS TO COLLABORATORS

Powder the sample in a mortar and dry at 110° C. Place 0.500 gram of the powdered tablets or 0.400 gram of the theocalcin powder, or 0.200 gram of theobromine alkaloid in a 300 cc. beaker and add 100 cc. of water. Warm moderately over a flame and add 15 cc. of approximately 0.1 N H_2SO_4. Heat to boiling to insure complete solution and to remove CO_2. Cool to room temperature under the tap. Add 1.5 cc. of phenol red indicator and render slightly alkaline with approximately 0.1 N NaOH, then titrate carefully to an acid reaction with 0.1 N H_2SO_4 (yellow color). To this solution add 25 cc. (an excess) of neutral 0.1 N $AgNO_3$ and titrate the liberated HNO_3 immediately with 0.05 N NaOH to a distinctly violet red color. Titrate cautiously drop by drop with constant stirring near the end point.

1 cc. of 0.05N NaOH = 0.009 gram of theobromine.

The results obtained are shown in the table.

COLLABORATOR	THEOBROMINE IN THEOBROMINE ALKALOID	THEOBROMINE IN THEOCALCIN POWDER	THEOBROMINE IN THEOCALCIN TABLETS
	per cent	*per cent*	*per cent*
M. L. Yakowitz Food & Drug Adm. San Francisco	99.8 –100.1	48.8 –48.5	41.2 –41.2
H. W. Gerritz Food & Drug Adm. San Francisco	99.48	48.79	41.8
I. S. Shupe Food & Drug. Adm. St. Louis	100.0 –99.9	48.58–48.65	41.46–41.41
S. Reznek Food & Drug Adm. New York City	99.7 – 99.9	48.4 –48.5	41.1– 41.3
P. S. Jorgensen Food & Drug Adm. San Francisco	99.63–100.08	48.69–48.46	41.47–41.47

COMMENTS BY COLLABORATORS

S. Reznek.—The method is simple and apparently accurate. My suggestion is that a comparison solution be used to determine the end point, which I found somewhat difficult to judge.

I. S. Shupe.—After boiling, the theobromine tends to crystallize out at the surface. With the theocalcin powdered tablets the sample did not wet readily. Would it be useful to moisten with alcohol? The end point in the titration faded a little. About 0.1 cc. additional alkali was added to obtain a permanent end point. A blank determination using 25 cc. of 0.1 N $AgNO_3$ used about 0.15 cc. of 0.05 N NaOH for a distinctly violet color.

CONCLUSIONS

The results indicate that this method gives an accurate measure of the theobromine in theocalcin powder, in theocalcin tablets, and in the alkaloid.

It is recommended[1] that the method be made tentative to supersede the previous method, and that the subject be further studied with the object of applying the method to the assay of theophylline and its salts.

REPORT ON CHLORBUTANOL

By F. C. Sinton (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

Work on chlorbutanol was continued during the current year, particularly with reference to collaborative study on the determination of chlorbutanol itself and development of a suitable method for the assay of chlorbutanol in such mixtures as occur in ampoule solutions, where it is extensively used as a preservative.

For purposes of experimental work and for use in preparing collaborative samples, a quantity of chlorbutanol labeled as anhydrous was purchased from a reputable manufacturer. This material was desiccated for two weeks, and at the end of that time it was found to pass U.S.P. tests for purity and have a melting point of 96° C. The material was allowed to remain in the desiccator except when used.

The previous report on this topic, *This Journal,* **19,** 535 (1936), indicated that satisfactory results for chlorbutanol itself could be obtained either by saponification with alkali in alcoholic solution or by applying a modification of the U.S.P. method for acetone. It was decided, therefore, to attempt to apply these methods to mixtures containing chlorbutanol. Since many of the preparations for which the method is intended would contain chlorides and iodine-consuming substances, experiments were carried out toward a separation of the chlorbutanol from the other constituents. For this purpose an aqueous solution was prepared containing 5 grams of the desiccated chlorbutanol per liter.

In the first attempt the solution was extracted with successive portions of ether, and to the combined ether extracts 25 cc. of 0.5 N alcoholic

[1] For report of Subcommittee B and action by the Association, see *This Journal,* **21,** 66 (1938).

potash was added. The mixture was thoroughly shaken, the ether was evaporated, and the chloride was determined. Maximum recovery of chlorbutanol in several determinations amounted to only 94 per cent of theoretical.

A method that gave promising results consisted in adding an excess of alkali and distilling off the acetone, which was subsequently determined in the distillate by a modified U.S.P. method. When this method was tested on a mixture containing procaine hydrochloride, however, the results were erratic and considerably higher than theoretical, amounting in one case to a recovery of 133 per cent. The divergent results were presumably caused by saponification of the procaine, with consequent distillation of some of the breakdown product resulting in adding to the iodine absorption.

It was found that chlorbutanol distils with water very readily. A material portion of the chlorbutanol sticks in the condenser, but this can readily be washed out with alcohol. As a result of this observation, a method was devised, involving first an aqueous distillation and then a hydroalcoholic distillation. The method gave reasonably close results on a known solution in the hands of the Associate Referee and Analyst J. C. Molitor and was then sent out to collaborators for study.

Two samples were submitted to collaborators: No. 1 consisted of chlorbutanol crystals, and No. 2 consisted of an aqueous solution containing in a liter 4.5 grams of chlorbutanol and 20 grams of procaine hydrochloride.

The method follows:

CHLORBUTANOL

REAGENTS

(a) *Alcoholic potassium hydroxide solution.*—Dissolve 30 grams of KOH in 30 cc. of water and add sufficient methyl alcohol to make 100 cc. Allow the solution to stand and when clear decant the liquid.

(b) *Silver nitrate solution.*—Dissolve 10 grams of $AgNO_3$ in sufficient water to make 500 cc.

DETERMINATION

Transfer to a pressure bottle a sample equivalent to about 0.4 gram of chlorbutanol and carefully add 25 cc. of the alcoholic KOH solution. Stopper, and mix the contents by gently swirling, then allow to stand 30 minutes. Place the bottle in a wire basket, and set the basket in a water bath at room temperature. Invert a tin can over the bottle and cover with a towel to prevent injury to the analyst in case the bottle should burst. Heat the bath to boiling and maintain at this temperature for 15 minutes.

Cool gradually; add 25 cc. of water, swirling gently, and transfer the contents of the pressure bottle to a 200 cc. volumetric flask. Wash the bottle out thoroughly with water, draining the washings into the flask. Bring to room temperature, fill to mark with water, and mix thoroughly.

Transfer a 50 cc. aliquot to a 500 cc. beaker, make acid with concentrated HNO_3 and add 5 cc. in excess. Add an excess of the $AgNO_3$, stir well, and allow the mixture to stand in a dark place for 15 minutes. Collect the precipitate upon a

Gooch crucible that has been previously prepared, dried at 105° C., and weighed. Wash the precipitate with several portions of distilled water, then with 5 cc. of alcohol followed by a 5 cc. portion of ether. Dry to constant weight at 105° C. If reagents contain chloride, apply the correction determined through a blank test.

1 gram of AgCl = 0.4127 gram of $C_4H_7OCl_3$

CHLORBUTANOL IN AMPOULE SOLUTIONS

For reagents see the preceding method.

Pipet into an alcohol distilling flask a sample equivalent to about 0.1 gram of chlorbutanol and add sufficient water to bring the volume to 50 cc. Distil about 25 cc. through a straight-bore condenser, collecting the distillate in a pressure bottle of approximately 100 cc. capacity containing 25 cc. of the alcoholic potash and surrounded by an ice bath. Allow the mixture to cool, and add to the distilling flask 25 cc. of alcohol. Continue distillation until 25 cc. more is distilled. (The volumes of distillate may be measured by previously marking the distilling flask.) Stopper the pressure bottle and mix the contents by gently swirling, then allow to stand 30 minutes. Place the bottle in a wire basket and set the basket in a water bath at room temperature. Invert a tin can over the bottle and cover with a towel to prevent injury to the analyst in case the bottle should burst. Heat the bath to boiling and maintain at this temperature for 15 minutes.

Cool gradually, and transfer the contents of the pressure bottle to a 500 cc. beaker, washing the bottle out thoroughly with water. Make acid with HNO_3 and add 5 cc. in excess. Add an excess of the $AgNO_3$, stir well, and allow the mixture to stand in a dark place for 15 minutes. Collect the precipitate upon a Gooch crucible that has been previously prepared, dried at 105° C., and weighed. Wash the precipitate with several portions of distilled water, then with 5 cc. of alcohol, followed by a 5 cc. portion of ether. Dry to constant weight at 105° C. If reagents contain chloride, apply the correction determined through a blank test.

1 gram of AgCl = 0.4127 gram of $C_4H_7OCl_3$.

The results follow:

	CHLORBUTANOL	CHLORBUTANOL IN SOLUTION (4 5 G./1000 CC)
	per cent	*g /1000 cc.*
R. L. Herd	98.88	4.20
Buffalo	98.94	3.97
	99.14	
E. H. Berry	99.13	4.20
Chicago	99.44	4.20
Paul S. Jorgensen	99.04	
San Francisco	98.92	
	99.45	
F. C. Sinton	99.2	4.43
	99.4	4.41
	99.4	4 38
L. E. Warren		4.33
Washington		4.16
J. C. Molitor		4.44
New York		4.37

COMMENTS BY COLLABORATORS

Jorgensen.—The method appears satisfactory and is not difficult to carry out.

Berry.—No difficulty was encountered.

Herd.—It seems as if a better precipitate would be obtained if the solution were boiled after the $AgNO_3$ is added. When these determinations were made, some AgCl remained in suspension and some of it passed through the filter.

CONCLUSIONS

The results by collaborators on chlorbutanol itself were reasonably satisfactory. It is believed that the difficulty in obtaining results closer to 100 per cent is likely due to the presence of a small quantity of water in the chlorbutanol, which appears to be difficult to remove. No explanation can be offered for the discrepancy in results obtained on the solution. There is apparently some detail of technic that needs clarification in order to obtain consistent results in different laboratories.

It is recommended[1] that the topic be continued for further study.

REPORT ON ASPIRIN AND PHENOLPHTHALEIN MIXTURES

By George M. Johnson (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

The work this year was a continuation of the method outlined in the previous report on this subject, *This Journal*, **20**, 598 (1937). A mixture of 9 per cent phenolphthalein and 76 per cent acetylsalicyclic acid with the common excipients, lactose, starch, talc, and calcium carbonate, was prepared and sent to the collaborators, who were requested to determine the phenolphthalein and the acetylsalicyclic acid by the following method:

Phenolphthalein.—Weigh accurately 1 gram of the powdered material and extract the dry sample with ether. Filter through paper into a separator. Test for complete extraction. Extract the ether solution with two 20 cc. portions of 4% $NaHCO_3$ solution, transferring the bicarbonate extractions to another funnel. Wash the ether with two 10 cc. portions of water and add to the bicarbonate solution. Filter the ether into a tared beaker, evaporate to dryness on the water bath, and dry the residue to constant weight at 105° C.

Acetylsalicyclic acid.—Acidify the combined bicarbonate and wash solution with concentrated HCl, using methyl orange as indicator. Add 1 or 2 drops in excess. Extract the liberated acetylsalicyclic acid with a 3:2 chloroform-ether mixture (30, 20, 20, 10, and 10 cc. fractions). Wash each extraction with 1 cc. (used for all extractions) of water in a separatory funnel and filter into a tared beaker. Test for complete extraction. Evaporate to a small volume on the water bath; remove and complete the evaporation spontaneously. Dry the residue to constant weight in a H_2SO_4 desiccator and weigh.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 66 (1938).

Five collaborative samples were sent out, and only three reports were returned. They are shown in Table 1.

TABLE 1.—*Collaborative results*

		PHENOLPHTHALEIN	ACETYLSALICYLIC ACID
		per cent	*per cent*
E. H. Berry	1.	9.40	35.46*
Food & Drug Adm.	2.	9.25	36.15*
Chicago	3.	9.26	45.80*
			54.13*
Jonas Carol	1.	9.03	74.98
Food & Drug Adm.	2.	9.22	74.89
Cincinnati	3.	9.26	74.57
H. G. Underwood	1.	9.22	75.65
Food & Drug Adm.	2.	9.22	75.21
Cincinnati			
Average:		9.22	75.06

* Not included in average

TABLE 2.—*Results obtained by Associate Referee with modified method*

NO.	4% $NaHCO_3$ PORTIONS	WASH WATER FOR ETHER (PORTIONS)	CHLOROFORM-ETHER WASHED?	PHENOL-PHTHALEIN	ACETYLSALI-CYLIC ACID
	cc.	*cc.*		*per cent*	*per cent*
1	20	10	No	9.04	75.82
	10	10			
2	20	10	No	8.85	75.73
	10	10			
3	20	10	Yes	9.12	75.49
	10	10			
	10				
4	25	5	No	9.23	75.24
	20	5			
5	25	5	No	9.12	76.21
	20	5			
6	20	10	No	9.65	75.13
	20	10			
Average:				9.17	75.60

COMMENTS BY COLLABORATORS

E. H. Berry.—Directions were followed exactly as described. Results show that a gravimetric determination for acetylsalicylic acid is not satisfactory, evidently due to hydrolysis. Suggest directions include determination by A.O.A.C. bromine method.

Jonas Carol.—Difficulty was experienced only once in the determination. That was in the first extraction of the sample with ether. Here it was necessary to make about 10 extractions in order to achieve complete extraction. Perhaps extraction from an aqueous suspension would hasten complete extraction.

Determinations made by the Associate Referee, using the same method with slight modifications, gave the results shown in Table 2.

Extraction from an aqueous suspension was tried by the Associate Referee, but less satisfactory results were obtained. The recovery of the acetylsalicylic acid was not so high. Ether is the best of the common solvents that will dissolve both substances. Phenolphthalein is not very soluble in any immiscible solvent. Berry's results indicate that some change is necessary in the method, perhaps the instruction, as the Associate Referee feels that the method is basically sound.

Because of the few collaborators and the discrepancies among them, it is recommended[1] that the subject be further studied and that samples again be sent to collaborators.

REPORT ON HOMATROPINE IN TABLETS

By E. M. Hoshall (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

The preliminary work on this topic carried out during 1935–1936 by the Associate Referee was divided for convenience into three parts, and the following report deals with the findings and the conclusions drawn in each of the three parts.

Part. I.—Assay of homatropine alkaloid and its salts in the commercially available pure substances.

An additional sample of homatropine hydrobromide and of homatropine alkaloid and two new samples of the hydrochloride were obtained from commercial sources and were tested for identity and purity by the U.S.P. XI method, with suitable modifications for the alkaloid and the hydrochloride. All products conformed to these tests in all particulars.

The above products were then examined by the method given in Part II. A 0.15 gram sample was used, and the procedure started with the second paragraph, reading, . . . "Dissolve in 10–20 cc. of water. . ." The results are given in Table 1.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 66 (1938).

TABLE 1.—*Experimental tests*

PRODUCT	TAKEN	FOUND	RECOVERY (PURITY)
	gram	*gram*	*per cent*
Homatropine hydrobromide (a)	0.1500	(Av. 4) 0.1488	99.20
Homatropine hydrobromide (b)	0.1500	(Av. 2) 0.1489	99.27
Homatropine hydrochloride (a)	0.1500	(Av. 2) 0.1495	99.67
Homatropine hydrochloride (b)	0.1500	(Av. 2) 0.1490	99 33
Homatropine alkaloid (a)	0.1500	0.1481	98 70
Homatropine alkaloid (b)	0.1500	0.1486	99.07

Part II.—Assay of homatropine in tablets.

As previously pointed out, practically all commercial tablets of this alkaloid and its salts are either dispensary, hypodermic, or ophthalmic types, and usually contain lactose as the tablet excipient.

For collaborative work, however, it was decided to submit a tablet mixture composed of the alkaloidal salt and a variety of tablet excipients, diluents, and a lubricant, in order to anticipate future use of these materials. The tablet mixture was selected for collaborative study principally because of the highly expensive nature of the alkaloid. It was also believed that a more homogeneous and better defined mixture could be obtained as a powder than as prepared tablets.

The collaborative mixture was prepared as follows:

U.S.P. homatropine hydrobromide (b), lactose, gelatin, talc, stearic acid, gum acacia, and a good grade of potato starch were pulverized separately, and passed through an 80-mesh sieve. Portions were then weighed as follows:

	grams		*per cent*
Homatropine hydrobromide	3.6	equivalent to	12 0
Lactose	12.0	equivalent to	40.0
Potato starch	4.8	equivalent to	16 0
Gelatin	3.0	equivalent to	10 0
Gum acacia	3.0	equivalent to	10.0
Talc	3.0	equivalent to	10 0
Stearic acid	0.6	equivalent to	2 0
			100.0

The material was thoroughly mixed by passing it several times through a 60-mesh screen, then placed in vials, sealed, and delivered to collaborators with the following instructions: "The sample consists of homatropine hydrobromide and suitable excipients simulating a commercial type of tablet mixture. Material sufficient for four determinations, 1 gram sample per determination, is submitted. The attached method should be used, starting with . . . 'transfer to a separator,' as in the first paragraph under Determination."

The submitted method was published in *This Journal*, 21, 95 (1938). The results obtained by collaborators are shown in Table 2.

TABLE 2.—*Collaborative results*

COLLABORATOR	NET TITRATION CC. 0.02 *N* ACID REQUIRED	HOMATROPINE HYDROBROMIDE FOUND	HOMATROPINE HYDROBROMIDE PRESENT	RECOVERY
		per cent	*per cent*	*per cent*
1.	16.77	11.94	12.00	99.50
	16.52	11.77	12.00	98.08
2.	17.39	12.39	12.00	103.25
	17.55	12.50	12.00	104.17
	17.34	12.35	12.00	102.92
	17.49	12.46	12.00	103.83
3.	16.93	12.06	12.00	100.50
	16.87	12.01	12.00	100.08
	16.87	12.01	12.00	100.08
	16.77	11.94	12.00	99.50
4.	16.96	12.08	12.00	100.67
	17.00	12.11	12.00	100.92
	16.92	12.05	12.00	100.42
	16.88	12.02	12.00	100.17
5.	16.76	11.94	12.00	99.50
	16.90	12.04	12.00	100.33
	16.82	11.98	12.00	99.83

COLLABORATORS

1. Donald C. Grove, Food and Drug Administration, Washington.
2. Wm. Reindollar, Bureau of Chemistry, State Department of Health, Baltimore.
3. Felice Rotondaro, Food and Drug Administration, Philadelphia.
4. The Associate Referee.
5. Solomon Reznek, Food and Drug Administration, New York.

Reindollar comments as follows:

A sharp separation of the immiscible solvents did not occur even when the material stood overnight. I found that the removal of the chloroform took place more efficiently without the aid of a fan. The latter has the disadvantage of keeping the liquid cool when chloroform, which is difficult to remove under any circumstances, remains in the form of globules at the bottom of the beaker.

Part III.—Assay of homatropine in the presence of cocaine.

A few commercially available ophthalmic preparations consist of mixtures of the hydrochlorides of homatropine and cocaine, and a diluent, usually lactose. Since the method described in Part II is not applicable

to this mixture, a method for the determinations of the alkaloids in such a mixture was sought.

The following conclusions are drawn from the various methods investigated:

1. No success was indicated with methods based upon the optical activity of cocaine (homatropine is inactive), due principally to the very small amounts of cocaine per tablet (usually about 0.04 grain per tablet), and consequently the large number of tablets required for an analysis (several hundred per determination).

2. An attempt was made to determine the benzoic acid resulting from the hydrolysis of the mixture of alkaloids, and it was found that homatropine was hydrolyzed simultaneously, and the resultant mixture of acids could not be assayed.

3. A wide variety of inorganic reagents was used in ascertaining their selective precipitation ability on salts of the two alkaloids. Alcoholic mercuric chloride gave some indication of this qualification, in that it was able to precipitate cocaine, and not react with homatropine. This reaction when studied in detail was found to be valueless from a quantitative standpoint.

4. As a final, and somewhat impracticable gesture to this problem, which by virtue of small sales and limited use of this mixture appears relatively unimportant, the following method was developed:

METHOD

Determine total anhydrous alkaloids by extraction and drying over H_2SO_4 for 24 hours. This weight will be represented by W.

To the aqueous residue remaining after the extraction of the alkaloids, add dilute HNO_3, transfer to a 100 cc. volumetric flask, and determine total chlorides by the Volhard method. Designate the net cc. of 0.1 N $AgNO_3$ as N. Then if x represents the amount of homatropine hydrochloride present and y the amount of cocaine hydrochloride, the following equations may be used:

$$(1)\quad 0.88297x+0.89262\,y=W$$
$$(2)\quad x/0.03116+y/0.03396=N$$

A series of determinations in which the utmost care was used both in drying the alkaloid and in titrations, failed to give results closer than 92 per cent of either alkaloid present.

This method is in general impracticable, and should only be used when the analyst will be satisfied with an approximate result.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method presented for determining homatropine in tablets be adopted as official, first action.

(2) That no additional study upon a method for the separation of cocaine from homatropine be made.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 67 (1938).

REPORT ON CUBEB

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York, N.Y.), *Associate Referee*

Work was continued in accordance with the recommendations approved last year. O. C. Kenworthy of this station and B. J. Thiegs of the North Dakota Regulatory Department collaborated.

Samples of coarsely ground cubeb were given to the collaborators, together with last year's report, *This Journal*, **20**, 602 (1937), and a method for the assay.

The results of assay are given in the table.

	Clevenger	*Kenworthy*	*Thiegs*
Resins (%)	9.06	9.78	9.3
Volatile oil (cc./100 g.)	18.2	18.6	18.3
Specific gravity (25°/25°)	0.917	0.917	0.916
Optical rotation*	−28.7	−36.3	—
Refractive index (20° C.)	1.494	1.493	1.495
Acid number	0.88	1.14	0.53
Ester number	3.8	4.9	5.9

* Angular degrees 25°, 100 mm. tube, white light.

The results are considered to be satisfactory. Variations in the results reported are probably accounted for by the variation in the elapsed time between grinding of the cubeb and the assay of the material.

It is recommended[1]

(1) That the method presented be adopted as tentative, and that the work on cubeb be discontinued for the present.

(2) That collaborative work be carried out on a method for the evaluation of cardamoms.

REPORT ON AMINOPYRINE AND PHENOBARBITAL IN MIXTURES

By E. C. PAYNE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

This investigation was confined, largely, to the determination of the most favorable conditions for the separation of aminopyrine from phenobarbital. The method consists in extracting the aminopyrine from a solution made alkaline with sodium hydroxide; the acidification of the residual aqueous layer, and the extraction of phenobarbital from it. Each substance was determined by weighing the residue, after evaporation of its solvent.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 67 (1938).

The experimental work included the determination of the best solvents, the number of extractions required, the most suitable concentration of alkali required; and the temperatures and times required for drying the extract. The data are summarized in Tables 1–4. In the experiments covered by this tabulated data, chloroform was used as the solvent for aminopyrine and ether for phenobarbital. From this material the following method was evolved:

METHOD

Aminopyrine.—Weigh sufficient powdered sample to yield 0.2–0.5 gram of aminopyrine and 0.05–0.3 gram of phenobarbital. Place in a separatory funnel and agitate with 10–20 ml. of 1 *N* NaOH. Extract the aminopyrine with $CHCl_3$, using 30, 20, 20, 20, and 10 ml. portions. (This should remove the aminopyrine completely.) Wash the $CHCl_3$ extract with 10 ml. of water, adding the washings to the aqueous layer. Filter the $CHCl_3$ into a tared beaker, evaporate on the steam bath, and dry for 2 hours at 80° C. Weigh as aminopyrine.

Phenobarbital.—Acidify the aqueous layer with HCl. Extract the phenobarbital completely with ether (six–eight 25 ml. portions are usually necessary). Wash the ether with 5–10 ml. of water acidified with HCl. Filter the ether into a tared beaker and evaporate on the steam bath, using a stream of air. Dry for 1 hour at 80° C. Weigh as phenobarbital.

The results obtained by the Associate Referee are given in the tables. No collaborative work was attempted.

The phenobarbital used in this investigation was Merck's U.S.P. grade, which was re-crystallized twice from chloroform containing a small quantity of alcohol. It melted at 175.5°–176° C. (corr.). The aminopyrine used was Merck's U.S.P. grade, cited(M. P. = 107°–108° C. corr.). A sample of this product, which had been re-crystallized from water, was used in some experiments, and gave yields essentially similar to those of the original material. Since the attempted purification yielded a product

TABLE 1.—*Results with 0.2 N NaOH*

	AMINOPYRINE			PHENOBARBITAL		
EXP. NO.	TAKEN	RECOVERY		TAKEN	RECOVERY	
	gram	*gram*	*per cent*	*gram*	*gram*	*per cent*
11	0.4032	0.4046	100.4	0.0972	0.0838	95 2
14	0.4543	0.4652	102.4	0.2104	0.2099	99.4
18	0.4019	0.4051	100.8			
19	0.4089	0.4220	103.2	0.2024	0.2004	99.0
24	0.4112	0.4120	99.8	0.2048	0.2123	103.7
			Average 101.3			Average 99.3
No. of Experiments		5		No. of Expts.	4	
Av. Recovery		101.3%		Av. Recovery	99.3%	
Range		99.8 −103.2%		Range	95.2 −103.7%	

yellow in color and melting at 106°–107° C., it was decided to use the original product, after drying.

In these experiments, the desired quantities of aminopyrine and phenobarbital were weighed separately for each experiment, and any added material, as lactose and starch, was added without any mixing prior to the addition of the alkali solution.

TABLE 2.—*Results with 0.5 N NaOH*

	AMINOPYRINE			NOTES	PHENOBARBITAL		
EXP. NO.	TAKEN	RECOVERY			TAKEN	RECOVERY	
	gram	*gram*	*per cent*		*gram*	*gram*	*per cent*
1	0.3033	0.3046	100.2		0.1995	0.1998	100.2
2	0.3230	0.3239	100.3		0.2039	0.1918	94.1
3				Aminopyrine lost	0.2012	0.1927	95.8
4	0.3007	0.2991	99.5	Phenobarbital lost			
7	0.3066	0.3068	100.1	Phenobarbital lost			
10	0.4151	0.4137	99.7		0.0847	0.0864	102.0
13	0.4087	0.4105	100.4		0.2063	0.2018	97.8
14				Aminopyrine lost	0.2104	0.2092	99.4
17	0.4289	0.4147	*96.7	*Omitted from average	0.2170	0.2155	99.3
20	0.4039	0.4063	100.6		0.2036	0.1994	97.9
23	0.4140	0.4140	100.0		0.2177	0.2150	98.8
			Average 100.1%				Average 99.3%
No. of Experiments			8		No. of Expts.		9
Av. Recovery			100.1%		Av. Recovery		99.3%
Range			99.5–100.6%		Range		94.1–102.0%

FURTHER STUDIES

Ether was tried as a solvent for aminopyrine, but so many extractions were required that its use was discontinued.

Previous work had been done by the writer on the titration of phenobarbital with 0.1 *N* sodium hydroxide, using thymolphthalein as indicator, by the method outlined by Morin,[1] and by Babich,[2]. With pure, recrystallized phenobarbital, results approximating quantitative precision were obtained. The titration of phenobarbital, extracted from aminopyrine, gave only about 95 per cent of the weighed residue, however. The work was not carried far enough to determine the cause of this discrepancy. Since preparations containing these two drugs usually have a large quantity of aminopyrine, with a small quantity of phenobarbital it seems impracticable to take a sufficient sample to have

[1] *J. Pharm. Chim.*, 21, 59 (1935).
[2] *Pharm. Monatsh.*, 17, 87 (1936).

enough phenobarbital to neutralize a desirably large titer of 0.1 N sodium hydroxide.

$$1 \text{ cc. of } 0.1N \text{ NaOH} = 0.02321 \text{ gram of phenobarbital.}$$

More dilute sodium hydroxide does not give a sharp end point. Therefore, it is doubtful whether this method would prove to be very useful. However, it is hoped that further work may be done on it.

TABLE 3.—*Results with 1N NaOH*

AMINOPYRINE					PHENOBARBITAL		
EXP. NO.	TAKEN	RECOVERY		NOTES	TAKEN	RECOVERY	
	gram	gram	per cent		gram	gram	per cent
12	0.4188	0.4162	99.4	Phenobarbital lost			
15	0.4023	0.4019	99 9		0.2070	0.2083	100.6
16	0.4021	0.4021	100.0		0 2197	0.2195	99.9
21	0.3992	0.4015	100.6		0 2082	0 2072	99.5
22	0.4061	0.4036	99 4		0 2124	0 2127	100.1
25	0.4198	0 4203	100.1	M.P. Phenobarbital = 171.5°- 172.5° (extracted)	0 2118	0 2119	100 0
26	0.4144	0.4140	99.9	Phenobarbital lost M.P. Aminopyrine = 107°–108° C. (extracted)			
27	0.4131	0.4125	99 9		0 2080	0.2074	99.7
28	0.4043	0.4073	100.7	M.P. Phenobarbital = 172°–175.5° C. (extracted)	0 2136	0.2128	99.6
29	0.4048	0.4045	99.9	0.1 g. each of starch and lactose added	0 2053	0 2049	99 8
30	0.4234	0.4234	100 0	Same as 29	0 2139	0 2120	99 1
			Average 100.0				Average 99 8
No. of Experiments			11		No. of Expts.		9
Av. Recovery			100.0%		Av. Recovery		99.8%
Range			99.4–100.7%		Range		99.1–100.6%

No attempt was made to determine phenobarbital by titration with silver nitrate, according to the method by Budde.[1]

A study was made to determine the required time and temperature of drying the extracted substances. These data are summarized in Table 4.

A few trials were made to find whether or not aminopyrine may be determined by alkalimetric determination, with 0.1 N acid. The indicators selected were 2, 4 dinitrophenol and methyl yellow (dimethylamino-

[1] *C.A.*, 28, 3176.

azobenzene), since they indicate change in approximately the *p*H range desired. The results were only roughly quantitative, due to lack of sharpness of the "end point." There was some indication that dinitrophenol is the more desirable. It is hoped that further work may be done on this method—perhaps by electrometric titration.

TABLE 4.—*Experiments on drying—successive heatings*

MATERIAL	ORIGINAL WEIGHT	80° C.				100° C.			115° C.
		WEIGHT (GRAMS) AFTER—							
		1 HR.	2 HRS.	4 HRS.	18 HRS.	2 HRS.	4 HRS.	18 HRS.	3 HRS.
	gram								
Aminopyrine pure, dry	0.2021		0.2020	0.2020	0.2007		0.1982	0.1923	
Aminopyrine extracted from phenobarbital and previously dried for 2 hrs. at 80° C.	0.2991				0.2917				
	0.4046				0.3989				
	0.4652	0.4600				0.4447			
	0.4215	0.4186							
	0.4115	0.4063							
Phenobarbital pure, dry	0.2009		0.2009	0.2010	0.2009				0.2007
	0.2025					0.2025	0.2022	0.2021	0.2019
Phenobarbital extracted from aminopyrine and previously dried 2 hrs. at 80° C.	0.0925				0.0838				
	0.2018					0.1997			
	0.2155							0.2056	
	0.0676					0.0511			
	0.1896							0.1783	
	0.0885					0.0430			(18 hrs.) 0.0197
	0.2004					(1 hr.) 0.1981			
	0.2072					(1 hr.) 0.2044			
	0.2127					(1 hr.) 0.2106			

DISCUSSION OF RESULTS

When 0.2 *N* sodium hydroxide was used in the separation, the recovery of aminopyrine was low, while that of the phenobarbital tended to be too high, indicating incomplete separation. Using either 0.5 *N* or 1 *N* sodium hydroxide, the yields were more nearly satisfactory. In the case of the 0.5 *N* solution, the percentages of recovery for phenobarbital were spread over a rather wide range (94.1–102.0 per cent), although the average figure was 99.3 per cent. The results with 1 *N* base (range 99.1–100.6 per cent) indicate that this is probably the most desirable concentration.

Under the optimum conditions, as determined above, this method appears to yield results of sufficient precision to warrant its use in analyzing mixtures of these two drugs.

Experiments 29 and 30 indicate that starch and lactose, common excipients in tablets, do not interfere. The removal of fatty acids with barium hydroxide by the method of Shupe, *Methods of Analysis, A.O.A.C.* 1935, **113**, p. 582, was not tested.

Table 4 shows that pure aminopyrine does not lose in weight appreciably when heated for 4 hours at 80° C. Longer heating at 80° C. and heating at 100° C., cause appreciable losses. Pure phenobarbital does not lose appreciably, even when heated for long periods at 100°, or even at 115° C. However, when these substances have been separated from a mixture, by extraction, serious losses seem to occur, even at 80° C., if the period of heating is protracted. At 100° even short heating causes loss.

It was very difficult to determine just how long to heat, and at what temperature. The decision to heat aminopyrine for 2 hours at 80° and phenobarbital for 1 hour at the same temperature was made after empirical study of the time required to dry the substance to a weight approximating that which was taken originally (after making sure that extraction was essentially complete). The melting points of the extracted substances, which were determined in a few cases (Experiments 25, 26, and 28) indicated that the substances were not entirely pure. The method, then, while yielding results that are fairly precise, empirically, leaves something to be desired in the matter of a clean separation of the two substances in the pure state.

SUMMARY

Conditions affecting the separation of aminopyrine from phenobarbital and the gravimetric determination of each were studied.

A method is outlined for the determination of these two substances when present in a mixture.

Empirically, the results seem to be of sufficient precision to warrant the use of the method in the anlaysis of pharmaceutical preparations.

There is some doubt as to whether or not a clean separation of the two substances in a pure state will be effected by this method.

REPORT ON EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE

By H. G. Underwood (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

The compound effervescent salt of the National Formulary was recommended for study this year. Investigational work was done to

develop methods for the quantitative determination of potassium bromide and caffeine.

Preliminary work by the Associate Referee showed that little difficulty would be encountered in the extraction of caffeine with chloroform. To ascertain whether the organic constituents would interfere with the direct determination of potassium bromide, the results obtained on samples from a preliminary laboratory batch on which the determinations were made directly were compared with results obtained on samples that were carefully carbonized in platinum and the carbonaceous mass leached with water. The results follow:

Potassium bromide

DIRECT	CARBONIZED
per cent	*per cent*
7.67	7.68
7.55	7.63 Av. 7.66
7.60	
7.63	
7.70 Av. 7.63	

The results indicate that removal of the organic material is not necessary. It was noted that if nitric acid was not present in definite excess the end point is indistinct.

The effervescent salt for collaborative work was prepared in the laboratory according to the general processes given in the N.F. VI, and granulated in an oven at 100° C. The final product was ground in a mortar, passed through a 60-mesh sieve, and intimately mixed. Samples, together with the proposed method, were submitted to collaborators. The method was published in *This Journal,* **21**, 96 (1938), but is repeated here because the quantities used have been reduced in some instances, and other suggested changes have been incorporated.

EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE

REAGENTS

(a) *Silver nitrate solution.*—0.1 *N*. Prepare and standardize as directed in *Methods of Analysis, A.O.A.C.*, 1935, 56, 87.

(b) *Ammonium or potassium thiocyanate solution.*—0.1 *N*. Adjust by titrating against the 0.1 *N* $AgNO_3$.

(c) *Ferric ammonium sulfate indicator.*—Dissolve 8 grams of $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ in sufficient water to make 100 cc.

(d) *Nitirc acid.*—Concentrated, C. P.

PREPARATION OF SAMPLE

Powder the sample, transfer immediately to a dry bottle, and seal tightly. Thoroly mix the powder in the bottle by rotating and shaking before removing the sample for analysis. Weigh out all needed portions as nearly at the same time as possible. Avoid extreme temperatures and humidities when opening and storing samples.

Collaborative results
(expressed as percentage)

COLLABORATOR	CAFFEINE 80° C.	CAFFEINE 100° C.	KBr ORIGINAL METHOD	KBr REVISED METHOD
Theoretical	0.798	0.798	8.30	8.30
Jonas Carol	0.782	0.780[1]	8.28	8.35
Cincinnati	0.784 Av. 0.783	0.774[1] Av. 0.777	8.25	8.32
			8.25 Av. 8.26	8.18[3]
				8.23[4]
				8.20[4] Av. 8.26
E. C. Deal	0.80	0.80	8.29[5]	
New Orleans	0.80	0.80	8.23[5]	
	0.80 Av. 0.80	0.80 Av. 0.80	8.17[5] Av. 8.23	
J. T. Field	0.782	0.775	8.23	8.26[5]
St. Louis	0.799 Av. 0.791	0.783 Av. 0.779	8.19	
			8.23[5]	
			8.16 Av. 8.20	
Maurice Harris	0.80	0.80	8.17	8.33
Chicago	0.79 Av. 0.795	0.79 Av. 0.795	8.07 Av. 8.12	8.41 Av. 8.35
Rupert Hyatt	0.792	0.785		8.26[5]
Cincinnati	0.789 Av. 0.791	0.787 Av. 0.786		8 26[5] Av. 8.26
		2 hrs. *Overnight*		
G. M. Johnson	0.783	0.782 0.765	8.09	8.32[5]
Minneapolis	0.787	0.791 0.777	8.17	8 27[5]
	0.788 Av. 0.786	0.793 0.775	8.24	8.30[5]
		Av. Av.	Av. 8.17	8.32[5]
		0.789 0.772		8.33[5]
				8.23[5]
				8.40[5] Av. 8.31
C. B. Stone	0.800	0.786	8.23	8.30
Cincinnati	0.797	0.785	8.22	8.30
	0.800 Av. 0.799	0.786 Av. 0.786	8.23 Av. 8.22	8.20 Av. 8.27
H. G. Underwood	0.789	0.780[2]	8.29	8.32
Cincinnati	0.786	0.781[2] Av. 0.781	8.24	8.29
	0.798		8.30	8.29
	0.801 Av. 0.794		8.35	8.26
			8.21	8.30
			8.33	8.33 Av. 8.30
			8.21	
			8.25 Av. 8.28	

[1] Still losing slightly at the end of 5 hours.
[2] Still losing slightly at the end of 4 hours.
[3] Determination made several days later than the above determinations.
[4] Determinations made from a second bottle of the salt.
[5] Weighings made by difference from a weighing bottle.

DETERMINATION

Potassium Bromide.—Weigh about 3 grams of the preparation and transfer to a 250 cc. Erlenmeyer flask. Add 50 cc. of water, avoiding loss of solution by spattering. Acidify the solution with the HNO_3 and then add 5 cc. in excess. Add 30 cc. of the $AgNO_3$ solution and 2 cc. of the indicator. Allow the mixture to stand several minutes and swirl occasionally as an aid in flocculating the AgBr. Titrate the excess of $AgNO_3$ with the thiocyanate solution. 1 cc. of 0.1 N $AgNO_3$ = 0.01190 gram of KBr.

Caffeine.—Weigh about 15 grams of the sample, transfer to a separator, and add slowly 50 cc. of water, avoiding loss of solution by spattering. If the solution is not alkaline to litmus, make basic with 5% NaOH solution. Add 50 cc. of $CHCl_3$, shake vigorously, and after clearing draw off the lower layer through a small filter, previously moistened with $CHCl_3$, into a beaker. Repeat the extraction twice, using 50 cc. portions of the $CHCl_3$ for each extraction. Wash the filter and funnel with a few cc. of $CHCl_3$ to remove any adhering caffeine. Evaporate the combined $CHCl_3$ filtrate on a water bath to about 10 cc., finally transferring the residual liquid by washing with $CHCl_3$, to a small weighed beaker. Allow the solution to evaporate by gentle heat, and an air blast. Dry the residue to constant weight at 80° C., and weigh as anhydrous caffeine.

COMMENTS OF COLLABORATORS

Jonas Carol.—The use of a weighing bottle did not seem to offer any advantage over direct weighing as the salt caked around the mouth on pouring.

G. M. Johnson.—It seems that heating the caffeine at 100° C. for short periods of time does not cause much loss by volatilization, but on protracted heating an appreciable amount of the alkaloid is lost.

E. C. Deal.—It is believed that a better method would be to weigh out a larger sample for bromide determination, make up to volume, and use an aliquot. To check upon this, a 15 gram sample was made to 500 cc. volume, and two separate 100 cc. aliquots were used for bromide determinations. The aliquots gave identical values of 8.24 per cent.

J. T. Field.—The humidity of the air was very high when I weighed my samples and it occurred to me that the hygroscopic nature of the mixture might warrant weighing the sample by difference from a weighing bottle. The error for moisture probably would be negligible, however, particularly in the 15 gram sample for caffeine.

Four collaborators found it necessary to weigh the samples for potassium bromide determinations by difference from a weighing bottle due to the relatively high humidity at the time of determination.

DISCUSSION OF RESULTS

As the result of a suggestion by G. M. Johnson, the collaborators, were requested to make determinations of potassium bromide, using 50 cc. of water to dissolve the sample. The Associate Referee believing also that 50 cc. of 0.1 N silver nitrate left too much in excess requested further that only 30 cc. be used. The collaborators and the Associate Referee found that the end point was more distinct at the lower dilution and in general found values that were more nearly in agreement with the theoretical value, even though the samples had been opened previously.

The method given previously in this report includes all the amendments suggested.

In view of the hygroscopic nature of the salt, the Associate Referee considers the results obtained by the collaborators on caffeine and potassium bromide to be very good. The chief cause of difference in results is no doubt the absorption of moisture, which is quite rapid if the humidity is at all high. The sample to be properly prepared should be free flowing after being ground in a mortar. If the powdered sample cakes so rapidly that it cannot be thoroughly mixed after being transferred to a well-corked bottle, the determination should be abandoned. The Associate Referee believes that if conditions can be met for the proper preparation of the sample, all weighings can be made directly from the sample bottle, even though some of the collaborators found it necessary to weigh the samples for potassium bromide by difference, due to the relatively high humidity at the time of determination. For the same reason the Associate Referee has not considered further the comment made by E. C. Deal.

In the determination of caffeine the results of collaborators indicate that there is possibly a slight loss of the alkaloid on drying at 100° C., therefore the method as originally written was changed to read, "Dry the residue to constant weight at 80° C."

In view of the results obtained it is recommended[1] that the method be adopted as tentative.

REPORT ON ELIXIR OF TERPIN HYDRATE AND CODEINE

By JONAS CAROL (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

Complying with last year's recommendation, a collaborative study of the analysis of elixir of terpin hydrate and codeine was made. Considerable work has been done on the determination of terpin in elixir of terpin hydrate, *This Journal*, **14**, 319 (1931), with the result that the method is now tentative, *Methods of Analysis, A.O.A.C.*, 1935, 563.

A method for the determination of both terpin hydrate and codeine has been suggested by the New York Station of the U. S. Food and Drug Administration (unpublished), and revisions of this method have been devised by A. E. Paul (unpublished). This year's work was undertaken in order to submit this method to collaborative study.

An elixir of terpin hydrate and codeine was prepared according to National Formulary VI, p. 129. The following method was submitted to the collaborators.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 67 (1938).

ELIXIR OF TERPIN HYDRATE WITH CODEINE

Terpin hydrate.—Pipet 10 cc. of the elixir (allow pipet to drain several minutes as liquid is somewhat viscous) into 10 cc. of water in a separator. Add 1–2 cc. of 10% H_2SO_4. Immediately extract (on standing crystals form and cause some inconvenience) with two 10 cc. portions of petroleum benzin and wash the combined petroleum benzin extracts three times with 2 cc. portions of water to which 3–4 drops of dilute H_2SO_4 have been added. (The petroleum benzin contains aromatics and may be discarded.) Return the acid washings to the original separator and extract completely with alcohol-$CHCl_3$ solution (7% alcohol) to remove the terpin hydrate. (Seven extractions with 20 cc. portions should be sufficient.) Make an additional extraction, evaporate to dryness, and test the residue for complete extraction. Wash each alcohol-$CHCl_3$ extract separately in a second separator with 7–8 cc. of 2% H_2SO_4. (This is very important since glycerin may be carried over. The washing also prevents loss of codeine.)

Filter the alcohol-$CHCl_3$ extract through a pledget of cotton, previously wet with alcohol-$CHCl_3$ into a tared beaker, and allow it to evaporate in a slow current of air. (Too rapid evaporation must be avoided, otherwise condensation of moisture will cause erroneous results.) After the residue is apparently dry let it stand in an air current for 1 hour and weigh. Weigh at intervals of 30 minutes to constant weight.

TABLE 1.—*Results on terpin hydrate and codeine*

COLLABORATORS	TERPIN HYDRATE		CODEINE
	ROOM TEMP.	50° C./10 MIN.	
	g./100 cc.	*g./100 cc.*	*g./100 cc.*
G. M. Johnson Minneapolis	1.700 1.716 1.721 Av. 1.707	1.691 1.705 1.706 Av. 1.701	0.195 0.195 0.195 Av. 0.195
H. G. Underwood Cincinnati	1.739 1.722 Av. 1.731	1.730 1.714 Av. 1.722	0.201 0.198 Av. 0.200
C. B. Stone Cincinnati	1.704 1.720 Av. 1.712	1.672 1.668 Av. 1.670	0.199 0.202 Av. 0.201
W. F. Kunke Chicago	1.762 1.755 1.740 Av. 1.756	1.759 1.755 1.730 Av. 1.748	0.195 0.192 0.194 Av. 0.194
R. Hyatt Cincinnati	1.746 1.720 Av. 1.733	1.739 1.708 Av. 1.723	0.200 0.198 Av. 0.199
S. M. Stark, Jr. St. Louis	1.752 1.798 Av. 1.775	1.729 1.769 Av. 1.749	0.190 0.189 Av. 0.190
O. C. Kenworthy New York	1.89 1.89 Av. 1.890	1.85 1.85 Av. 1.850	0.203 0.206 Av. 0.205
J. Carol Cincinnati	1.700 1.696 1.710 Av. 1.702	1.692 1.683 1.695 Av. 1.690	0.202 0.200 0.200 0.199 Av. 0.200

Codeine.—Transfer the acid wash material to the original separator and make the solution alkaline with ammonia T. S. Determine codeine by the A.O.A.C. method, *Methods of Analysis, A.O.A.C.*; 1935, 565, 69, beginning line 4 "... extract five times with $CHCl_3$ ...."

$$1 \text{ cc. of } 0.02\ N\ H_2SO_4 = 0.00634 \text{ g of } C_{18}H_{21}O_3N \cdot H_2O$$

Table 1 contains the results obtained by the collaborators and the Associate Referee.

The results indicate that good recovery of the codeine can be accomplished despite the small (approximately 3 cc.) titration.

Several of the collaborators obtained high results for terpin hydrate. This was undoubtedly due to condensation of moisture during evaporation of the chloroform-alcohol extract. The Associate Referee experienced this difficulty in several determinations and obtained very high results.

The heating of the residue of terpin hydrate for 10 minutes at 50° C. was tried in order to dry those samples that had absorbed water during evaporation. Collaborative results showed this heating did not prove successful in removing the excess moisture. Continued heating even at 50° C. caused a constant loss of weight until the terpin hydrate lost water of hydration.

In view of the results obtained, the Associate Referee recommends[1]—

(1) That the proposed method, with heating of the terpin hydrate residue at 50° C. deleted, be adopted as a tentative method.

(2) That further work be done to try and find a more accurate and uniform method of drying the terpin hydrate.

REPORT ON EMULSIONS OF COD LIVER OIL

By W. F. KUNKE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

A study of this subject has not been undertaken previously by the Association. The work this year was limited to the experimental study of various chloroform extraction procedures by which it was thought an accurate, rapid, and reliable method for the quantitative determination of cod liver oil could be developed. Only samples of the Emulsion of Cod Liver Oil, U.S.P., were used; no attention was given to the N. F. emulsions that contain cod liver oil. Collaborative work has not been undertaken and no method is proposed.

PROCEDURES TRIED

Wet extraction or the extraction of a sample of emulsion of cod liver oil after the addition of various proportions of water in a separator with

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 67 (1938).

chloroform gave troublesome emulsions even when shaken gently. Extraction of the comparatively dry uniform mixture of the sample and powdered pumice was tried in a thimble with chloroform by means of a Soxhlet extraction apparatus. The chloroform was filtered into a tared beaker and evaporated slowly on a steam bath by a slow current of air to avoid condensation of water in the beaker, and the residue was weighed. The samples of the emulsion contained from 1.8117 to 3.6860 grams of cod liver oil, and the quantity of powdered pumice added was 20 or 30 grams. The results obtained when the extraction period was carried out with siphoning every 5 minutes were as follows:

EXP.	EXTRACTION PERIOD	COD LIVER OIL RECOVERY
	hours	*per cent*
1	2½ after standing overnight with chloroform	68.0
2	4½ —	79.0
3	4½ —	96.5
4	4½ Exp. No. 3 continued (total)	97.4
5	1 hr. followed by standing overnight with chloroform and 2 hrs. more extraction	98.2

The U.S.P. formula for emulsion of cod liver oil specifies 10 per cent, by volume, of sirup. It was suspected that the sirup might retain a small quantity of the oil or markedly delay the complete extraction with chloroform. Accordingly, a sample of an emulsion (without the sirup ingredient) containing 2.0030 grams of cod liver oil was mixed thoroughly with 20 grams of powdered pumice and extracted as described previously for 4.5 hours with the result of 100.6 per cent. This finding indicates that the sirup may have some retarding effect on the extraction.

The extraction of the emulsion of cod liver oil-pumice mixture in a beaker with chloroform gave a result of 99.8 per cent. Six portions of 100 cc. each of chloroform were used for extraction during 2 hours, the solvent being pressed out by means of a glass rod having a disc-like end about three-fourths of an inch in diameter, which was necessary because the pumice mixture, by simple decantation, retained considerable chloroform. As in the other procedure, the chloroform was filtered through a filter paper and evaporated, and the residue was weighed.

The Soxhlet extraction procedure was discarded because complete recovery was not obtained even after a long extraction period coupled with frequent siphoning. The extraction of the emulsion-pumice in a beaker required large volumes of chloroform.

DETAILS TO BE STUDIED

It would seem that some satisfactory material to break the emulsion or to be used as a "spreader" may be found so that the cod liver oil can be more readily extracted.

Emulsifiers, other than acacia—namely, agar, gelatin, or tragacanth—

are permitted in the U.S.P., but it is believed that if a method is reliable for an emulsion that contains acacia it will be satisfactory for the emulsions prepared by the use of the emulsifiers named.

Also it would be desirable to know if the method, which may be proposed, is applicable to the three N.F. emulsions, which contain from 30 to 50 per cent, by volume, of cod liver oil, and certain other ingredients; namely, hypophosphites, egg yolk, and extract of malt besides from 7–9 per cent of alcohol. Furthermore, glycerol is an ingredient of some cod liver oil emulsions on the market.

An essential requirement of a method is that sufficient cod liver oil, extracted from the sample, should be available in case the physical constants are to be determined to confirm possible adulteration.

Before a method is proposed and collaborative work undertaken it appears desirable to study certain other details, (1) the necessity, if any, for blanks on the materials used, (2) the possible oxidation of the oil during extraction, evaporation of the solvent, and drying of the sample to constant weight, and (3) the effect, if any, of the procedure on the physical constants of the cod liver oil.

CONCLUSION

Some of these details have been studied experimentally and considering the subject as a whole, good progress has been made.

With the experience gained this year and further study, it is believed that the Associate Referee will be able to submit a method for collaborative work and recommend an accurate method next year.

Accordingly, it is recommended[1] that work on this subject be continued.

REPORT ON OINTMENT OF MERCURIC NITRATE (CITRINE OINTMENT)

By H. O. Moraw (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Citrine ointment is official in the National Formulary, but no method of assay is provided. It is prepared by mixing a solution of 7 grams of mercury dissolved in 10 grams of nitric acid with the product resulting from heating 76 grams of lard with 7 grams of nitric acid. Three batches prepared in the laboratory were lemon yellow in color, and seemed to be of suitable consistency at temperatures of approximately 78° F. and above, but were too stiff at lower temperatures. Samples representing the output of pharmaceutical manufacturers found in trade channels are

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 67 (1938).

dark brown in color and softer in consistency than those prepared by the Associate Referee.

Of the various methods of assay for mercury in ointments, those selected as most suitable for citrine ointment were the gravimetric or mercuric sulfide[1] method and the titration method with standard solution of thiocyanate.[2] The time consumed in the sulfide method for washing the precipitate free from sulfur and the intermediate drying is frequently too long for routine analyses. However, the method could be applied to this product.

The thiocyanate method with suitable treatment for separating the mercury from the ointment base is official in the U.S.P. XI for strong and mild mercurial ointments as well as for the assay of mercury itself. Authorities are agreed that mercurous salts[2] and chlorides or hydrochloric acid[3] and certain other elements must be absent when the thiocyanate method is used. In this connection the method proposed by Wright[4] was tried by the Associate Referee, who applied the thiocyanate titration to samples of citrine ointment of known mercury content which had been oxidized with perchloric and nitric acids. The end points in the titration were as indefinite and unsatisfactory as when the untreated solution resulting from digesting the sample with nitric acid and water, 1 to 1, was titrated, which procedure was modified because of unsatisfactory end points. The results of the above assays on the known sample are shown in Table 1.

Since this preliminary investigation appeared to indicate that the presence of oxides of nitrogen or monovalent mercury or in the case of the Wright method probably chlorides were the cause of unsatisfactory end points in applying the thiocyanate method, efforts were made to apply the acid potassium permanganate treatment[3,5] to the filtered nitric acid solution. This treatment solved the problem of indefiniteness of end point and when properly applied yielded correct results. The method in preliminary form was then submitted to collaborators K. L. Milstead and E. H. Berry at the Chicago Station of the Food and Drug Administration. Their first results were low. Further assays by Milstead, using more permanganate, yielded higher results but indicated either insufficient permanganate or time of treatment therewith. A solution representing half of a sample assayed by the Associate Referee was then submitted to Milstead with revised directions for the permanganate treatment. Berry applied the method to the collaborative samples according to the revised instructions. Agreement in results was then obtained. The results are reported in Table 1.

[1] J. Volhard, *Liebig's Ann.*, **255**, 255 (1889).
[2] E. Rupp, *Chem Ztg*, **32**, 1077 (1908).
[3] Hillebrand and Lundell, Applied Inorganic Analysis, 1929, 173.
[4] *J Am. Pharm Assoc.*, **24**(2), 102 (1935).
[5] Rupp and Nöll, *Arch. Pharm.*, **243**, 1-5 (1905).

TABLE 1.—*Investigational results and preliminary collaborative results*

ANALYST	SAMPLE	METHOD OF DISSOLVING	METHOD OF DETERMINATION	Hg PRESENT	Hg FOUND	COMMENT
				per cent	*per cent*	
H.O.M.	Known	Approx. 1 hr. digestion HNO_3 (1+1)	Thiocyanate titration of untreated filtrate	7.58	7.0	End point indefinite
H.O.M.	Known	do	do	7 58	7.52	do
H.O.M.	Known	Same as above except added 5 cc. conc. H_2SO_4	do	7 58	7 69	End point faded
H.O.M.	Known	do	do	7.58	7 97	do
H.O.M.	Known	Approx. 1 hr. digestion HNO_3 (1+1)	Filtrate neutralized by NH_4OH. Added 5 cc. HCl. pptd as HgS	7.58	7.66 7.69	Method satisfactory but too long
H.O.M.	Known	do	Thiocyanate titration without further treatment	7.58	8.62	End point faded, indefinite
H.O.M.	Known	Approx. 1 hr. digestion HNO_3 (1+1)+4 cc. $HClO_4$	½ of filtrate neutral to NH_4OH acid by HNO_3 titrated with thiocyanate.	7 58	8 29	do
H.O.M.	Known	do	½ of filtrate titrated without further treatment	7.58	9 53	End point faded
H.O.M.	Known	HNO_3 (1+1) round-bottomed Kjeldahl digested 1¼ hrs.	Acid permanganate treatment of filtrate, titration with thiocyanate	7.58 7 58	7 65 7 40	End point satisfactory
H.O.M.	Known	do	do	7.53	7.57	do
H.O.M.	Known	do	do	7.53	7.52	do
H.O.M.	Known	do	do	7.53	7.49	do
H.O.M.	Known	do	do	7 53	7.39	do
H.O.M.	Commercial	do	do	Unknown	7.30	do
H.O.M.	Commercial	do	Determination completed as HgS	do	7.39 7.53	Results high

TABLE 1.—(*Continued*)

ANALYST	SAMPLE	METHOD OF DISSOLVING	METHOD OF DETERMINATION	Hg PRESENT	Hg FOUND	COMMENT
				per cent	*per cent*	
K.L.M.	Known	do	Thiocyanate titration after permanganate treatment	7.53	6.74 6.63	Low results due to insufficient permanganate
K.L.M.	Known	do	Thiocyanate titration increased amount of permanganate	7.53	7.36 7.15	do
K.L.M.	Known	do	Thiocyanate titration after longer digestion with permanganate	7.53	7.54	
K.L.M.	Commercial	do	do	Unknown	7.31	
E.H.B.	Known	do	The method before revision	7.53	7.26 7.08	

PURITY OF MERCURY AND MERCURY CONTENT OF COLLABORATIVE SAMPLE

The mercury used in preparing the collaborative samples was "redistilled mercury" and was found by assay to be a practically pure product. The theoretical mercury content of the two samples prepared in the laboratory was 7.58 and 7.53 per cent, respectively.

The investigational results of the Associate Referee and the preliminary collaborative results of K. L. Milstead and E. H. Berry are also given in Table 1.

Tests for complete extraction or separation of the mercury from the ointment base were made by the Associate Referee on the residues of several samples that had been treated as directed in the method. It was found that the nitric acid solutions from the second treatment, when subjected to the acid permanganate treatment, and titrated with 0.1 *N* thiocyanate, gave a distinct color from the indicator with not more than 1 to 2 drops of thiocyanate.

The collaborative samples with the essentials of the method as given below, but with slightly different wording for the permanganate treatment, were submitted for trial to other collaborators. The results are given in Table 2.

TABLE 2.—*Collaborative results on citrine ointment*

NAME OF COLLABORATOR	SAMPLE A Hg PRESENT 7.53%	SAMPLE B Hg PRESENT UNKNOWN
	per cent	*per cent*
K. L. Milstead	7.48	7.34
U. S. Food & Drug Adm., Chicago	7.45	7.31
E. H. Berry	7.49	7.23
U. S. Food & Drug Adm., Chicago		
Wm. F. Reindollar	7.35	7.13
State Depart. of Health, Baltimore	7.34	7.20
S. Reznek	7 26	7.11
U. S. Food & Drug Adm., New York	7.38	7.01
L. E. Warren	7.11	6.71
U. S. Food & Drug Adm., Washington	7.15	6.90
Rupert Hyatt	7 40	7.30
U. S. Food & Drug Adm., Cincinnati	7.51	7.31
C. B. Stone	7 26	7 15
U. S. Food & Drug Adm , Cincinnati	7.19	7.06
E. H. Grant		7.24
U. S. Food & Drug Adm., Boston	6 99	7.39

DISCUSSION OF RESULTS

Correct results on the known sample by several collaborators support the conclusion reached by the Associate Referee that the method is dependable and accurate. The low results obtained by some collaborators are attributed to fault in the details of the directions sent to them with respect to the permanganate treatment. The method now submitted has accordingly been reworded on this point so that there should be no chance of failing to have a definite excess present.

The mean of 14 results reported by collaborators on the known sample prepared by the Associate Referee is 7.31 per cent compared with 7.53 per cent mercury present, and the mean of 15 results on the commercial sample is 7.16 per cent. The highest result on the known sample is 7.51 per cent and the lowest is 6.99 per cent. The difference, 0.52 per cent, is 6.9 per cent of the theoretical. It is confidently believed that results within 0.1 per cent of the correct amount can be obtained by applying the revised directions. Since only about 3 hours for an assay is required by this method compared with 6–12 hours or more for the gravimetric method, which is less accurate, the proposed method should be chosen.

Experience in procuring samples of citrine ointment in trade channels indicates it is at present produced by a small number of manufacturers. If this properly reflects the extent of its use, the subject does not warrant further study.

The method follows:

MERCURY IN OINTMENT OF MERCURIC NITRATE (CITRINE OINTMENT)

Weigh accurately 2–3 grams of the sample on a small piece of glassine paper, using a glass or bone spatula. Place in a 500 cc. round-bottomed Kjedahl flask and insert a small funnel in the neck of the flask. Add glass beads and 40 cc. of HNO_3 (1+1), place the flask in an upright position, and boil gently over a low flame. (Place a piece of asbestos having a circular hole under an asbestos wire-gauze, or use a sand bath.) Boil the contents of the flask for 1–1.5 hours, then add 30–40 cc. of water, using part to wash down the funnel. Cool, and filter (using an 11 cm. filter) into a 200 cc. volumetric flask. Wash the fat, flask, and filter, using approximately 100 cc. of 1% HNO_3. Make to volume and mix well.

Transfer a 100 cc. aliquot to a 500 cc. Erlenmeyer flask and add 7 cc. of HNO_3 and 5 cc. of H_2SO_4. Place the flask over the flame or on hot plate and add small amounts of $KMnO_4$ crystals until a definite excess is indicated by a dark purple color that persists during 3–5 minutes while boiling gently (a total of 2–5 grams may be required). Place on a steam bath and add more permanganate crystals if needed, to give a dark purple color persisting for 15 minutes while on the bath. Remove excess permangante and dissolve MnO_2 by adding H_2O_2 dropwise to the hot solution. Remove from the bath and add $KMnO_4$ solution until a faint pink persists for about 1 minute. (Only a small amount of MnO_2, if any, should form at this point. If a large amount forms, repeat the procedure, adding more permanganate crystals and digesting.) Discharge the color from the last permanganate treatment by adding dropwise sufficient $FeSO_4$, T.S. Cool to about 20° C., add 3 cc. of ferric ammonium sulfate indicator and titrate with the standard thiocyanate, 1 cc. of 0.1 *N* ammonium thiocyanate = 0.01003 gram of Hg.

SUGGESTIONS BY COLLABORATORS

K. L. Milstead.—My work on citrine ointment during the development of the method and the assay of the collaborative samples indicates the following: The directions in the original method were inadequate since I obtained check results but they were about 1% lower than those of the Associate Referee. Subsequent analyses in which a large excess of permanganate was insured by frequent additions to maintain a dark purple color, which persisted through the preliminary boiling as well as one-half hour in the steam bath, yielded still higher results, which so far as could be determined showed the maximum mercury content. If these conditions are maintained, the method seems to be satisfactory.

Rupert Hyatt.—Reasons for difference in results by Collaborator Stone and me (in same laboratory) are not apparent. My solutions were of smaller volume than his. I used less permanganate. The conditions of heating may be the important factor as we checked ourselves but not each other. The fat may occlude some mercury, which could be removed by subsequent hot washing. It does not appear necessary to shake the flask during digestion.

(The method submitted by the Associate Referee is largely the reworded method as suggested by Hyatt.)

RECOMMENDATIONS

It is recommended[1]—

(1) That the method as revised to insure excess permanganate, and submitted in this report, be adopted as a tentative method.

(2) That the subject be considered closed.

The proposed method was tried by the Associate Referee on a sample of mercuric oxide ointment and found to work satisfactorily. It therefore appears that it would be advantageous with respect to time saving and accuracy over the gravimetric mercury sulfide method, which is usually employed for the red and yellow mercury oxide ointments.

REPORT ON RHUBARB AND RHAPONTICUM

By Elmer H. Wirth (University of Illinois College of Pharmacy, Chicago, Ill.), *Associate Referee*

The inferiority of rhapontic rhubarb as compared with Chinese rhubarb is a well established fact. It is because of this inferiority that rhapontic rhubarb is denied entry at United States ports, and a concerted effort is being made in all directions to prevent the adulteration or substitution of Chinese rhubarb with it. Unfortunately the botanical source of the rhubarbs is hard to control and their chemistry, in spite of much study, is by no means complete. These and other aspects of the problem are discussed by Viehoever in a previous report, *This Journal*, **16**, 527 (1933). The problem of finding means for the detection of adulteration and substitution of Chinese rhubarb with rhapontic rhubarb is therefore still an open one.

CHEMICAL

The United States Pharmacopoeia X included a test for rhapontic rhubarb in which an alcoholic extract of the rhubarb is concentrated, treated with ether, and allowed to stand. In the event of the presence of rhapontic rhubarb, rhaponticin crystallizes out. With mixtures containing equal parts of Chinese and rhapontic rhubarb the crystallization takes place in about 24 hours; mixtures containing 25 per cent of rhapontic rhubarb require a longer time and with mixtures containing less than 25 per cent rhapontic rhubarb the test is unreliable. Because of this unreliability the test was not included in the United States Pharmacopoeia XI, although it does still appear in certain foreign pharmacopoeias.

A few microchemical methods have been suggested, but these have not received verification. Color tests should be thoroughly investigated and tests depending upon microsublimation and microcrystallization should be attended by a careful microcrystallographic examination. The limited

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **21**, 67 (1938).

knowledge of crystallographic methods among chemists today probably accounts for the lack of development in this direction.

MORPHOLOGICAL AND HISTOLOGICAL

While some slight morphological differences between Chinese and rhapontic rhubarb exist, they are by no means constant and only experts who handle quantities of both are able to distinguish between them by the usual organoleptic means. Histologically, their close similarity again prevents any definite means of differentiation based upon structural elements. Powdering a drug destroys all of its morphological characters and most of its anatomical characters, thus leaving cell contents and fragments of tissues as the only means of identification. Since adulteration will occur mostly in the powdered state and since there is no distinct microscopical difference between the powders of these two drugs it may safely be said that differentiation by morphological and histological means shows no promise.

FLUORESCENCE

Other than the possible development of a satisfactory chemical test the most promising field of endeavor seems to be that of observation in filtered ultraviolet light. Maheu[1] was the first to observe that rhapontic rhubarb exhibited a definite violet fluorescence in filtered ultraviolet light while Chinese rhubarb exhibited but a slight velvety reddish-brown fluorescence. Maheu claimed to be able to detect and approximately estimate any amount not less than 10 per cent of rhapontic rhubarb in mixtures. This has been more or less verified by other workers.

In 1934 Wallis and Withell[2] devised a method in which they prepared a tincture of the rhubarb sample under investigation. Non-fluorescing wood-cellulose paper was then impregnated with the tincture and examined in filtered ultraviolet light. Using genuine Chinese rhubarb for comparison, these authors report their ability to detect as little as 1 per cent of added rhapontic rhubarb. Among other things, these authors found that the violet fluorescence of rhapontic rhubarb is gradually destroyed under the influence of continuous exposure to ultraviolet light and more slowly on exposure to daylight. Ultimately a yellow fluorescence remains. Exposure to a temperature of 70° C. for 18 hours markedly diminished the violet fluorescence of rhapontic rhubarb, and after the temperature had been raised to 100° C. for 100 hours a further reduction in the intensity of the fluorescence was observed. A wide difference in the intensities of the fluorescence of different samples of rhapontic rhubarb was noted and for this reason it was difficult to carry out any accurate quantitative determinations. The preparation of the tincture as well as

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[1] *Bull. Sci. Pharmacol.*, 35, 278 (1928).
[2] *Quart. J. Pharmacol.*, 7, 574 (1934).

the impregnation and drying of the wood-cellulose paper should be carried out in the dark.

In 1936 Crews[1] studied this method further and also investigated its adaptability in the detection of rhapontic rhubarb in galenical rhubarb preparations. He employed washed cotton as the cellulose fiber. This was placed in a nonfluorescent glass tube through which the tincture was allowed to percolate. The cotton was then washed with water to remove the yellow coloring matter and examined (in the tube) in filtered ultraviolet light. It should be noted that the tincture must not be filtered due to the adsorption of the fluorescent principle by the filter paper. Crews reports his ability to detect rhapontic rhubarb in mixtures containing 0.1 per cent by this "adsorption-fluorescence" method. Rhapontic rhubarb could be detected with ease in galenicals. He also made an attempt to isolate the fluorescent principle, and in this connection found that rhaponticin (obtained in the usual way) gave a marked fluorescence, detectable in dilutions of 1–25,000,000. Spectrophotometric examination showed selective absorption in the ultraviolet region of the spectrum at 320 mμ.

THE PROBLEM

Before a suitable test for rhapontic rhubarb can be evolved further studies along chemical lines are necessary: microchemical investigations should be carried out on rhaponticin and other crystalline sublimates and extractives with special reference to their crystallographic constants and the fluorescence tests should be investigated. The work has been considerably hampered during the past year by the inability of obtaining authentic material for study. A possible supply now seems to have been located, and difficulties attending its importation have been removed. The laboratory of the Associate Referee has recently added a Hanovia analytical quartz lamp to its equipment. The laboratory also has a petrographic microscope and other necessary apparatus. As soon as a supply of rhapontic rhubarb can be obtained it is hoped that studies can be carried out covering all three divisions of the problem.

It is recommended that the topic be continued.

REPORT ON THEOPHYLLINE SODIUM SALICYLATE

By M. Harris (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In accordance with the recommendations made last year, investigational work was undertaken to devise a quantitative method for the determination of theophylline sodium salicylate.

[1] *Quart. J. Pharmacol.*, 9, 434 (1936).

This product is not included in the U.S.P. or other recognized publications, hence no authentic information as to its composition is available. A product, "theophylline-sodium salicylate," is described in Merck's index, 4th ed., p. 519, as $C_5HN_4O_2(CH_3)_2\cdot Na\cdot C_6H_4OH\cdot COO\ Na$. It is noted that this product has no water of crystallization.

In the preliminary work it was found that ethyl alcohol could be substituted for isopropyl alcohol in the solvent specified for the extraction of theophylline in the tentative A.O.A.C. method. Comparative results are shown in Table 1.

TABLE 1.—*Comparison of results obtained with isopropyl alcohol and ethyl alcohol*

WT. OF SAMPLE	METHOD	ANHYDROUS THEOPHYLLINE FOUND	THEOPHYLLINE $\cdot H_2O$ EQUIVALENT	RECOVERED*
gram		*gram*	*per cent*	*per cent*
0.2677	Tentative A.O.A.C. Method, with Chloroform and Isopropyl Alcohol	0.2456	100.9	99.39
0.2930	"	0.2679	100.5	99.11
0.2727	Tentative A.O.A.C. Method, with Chloroform and Ethyl Alcohol	0.2498	100.7	99.28
0.2509	"	0.2300	100.8	99.35

* Based on correction for partial loss of water of crystallization H_2O found $=7.75\%$.

Attempts to determine theophylline in combination with sodium salicylate by the U.S.P. assay for theophylline or theophylline sodium acetate were unsuccessful due to the fact that methyl salicylate was formed in the methylation procedure. The Stevens and Wilson method,[1] based on the formation of the silver salt of theophylline in ammoniacal solution and titration of the unconsumed standard silver nitrate solution, was likewise unsatisfactory when used directly, since the end point was masked by the salicylic acid on addition of the ferric alum indicator. Accordingly, it was deemed necessary to develop a procedure effecting a complete separation of the components.

As a basis for this study, specimens containing definite proportions were prepared. The material consisted of a mixture containing three parts of theophylline U.S.P. (equivalent to 2.768 parts of anhydrous theophylline on the basis of its water content of 7.75 per cent) and four parts

[1] *J. Am. Pharm. Assoc.*, 26, 314 (1937).

of sodium salicylate U.S.P., which assayed 100.2 per cent by titration and 99.7 per cent by the A.O.A.C. bromine method.

Two methods were submitted for collaborative study. The first is a gravimetric method in which the salicylate is removed by means of a solvent consisting of carbon tetrachloride and ether and determined by the A.O.A.C. bromine method. The theophylline in the remaining acid solution is extracted with chloroform-ethyl alcohol solvent and weighed. The volumetric method is a modification of the Stevens method, in which theophylline is determined by titration of the unconsumed silver nitrate in the filtrate, which is previously shaken out with chloroform-ethyl alcohol solvent to remove the salicylic acid.

The material submitted for collaborative study consisted of Sample 1, which contained three parts of theophylline and four parts of sodium salicylate, as used by the Associate Referee in the preliminary work, and Sample 2, which contained five parts of the commercial product theophylline sodium salicylate, one part of starch and one part of lactose. Analysis of the commercial product alone by the proposed methods indicated 42.64 per cent and 42.44 per cent of anhydrous theophylline.

The methods are as follows:

THEOPHYLLINE SODIUM SALICYLATE

Gravimetric Method

PREPARATION OF SAMPLE

Weigh from 0.6–0.7 gram of the material into a separator and add 5 cc. of 2% NaOH solution. Shake gently to complete solution.

EXTRACTION OF SALICYLIC ACID

Add in the order named 3 cc. of water, 50 cc. of CCl_4-ether solvent (9+1) and sufficient HCl (1+3) dropwise until acid to litmus, then 1 cc. in excess. Shake for 1 to 2 minutes and allow to settle. Transfer the lower layer into a second separator, which contains 10 cc. of water and several drops of the dilute HCl. Shake well, allow to settle, and transfer the lower layer into a third separator. Test for complete precipitation of the salicylic acid by adding a few drops of the HCl (1+3) to the first separator. Repeat the extraction with a 40 cc. portion, followed by three 20 cc. portions of the CCl_4-ether solvent, washing each portion through the second separator and collecting the combined portions in the third separator. Wash the combined solvent with two 5 cc. portions of water acidified with three drops of HCl (1+3) and add the washings to the main aqueous solution in the first separator. Determine salicylic acid by shaking the CCl_4-ether solvent with three 20 cc. portions of 2% NaOH solution and two 20 cc. portions of water. Combine alkaline extract and washings in a 200 cc. volumetric flask and make to mark. Proceed as directed in *Methods of Analysis A.O.A.C.*, 1935, 551, **27**, beginning with "Transfer an aliquot . . . ".

EXTRACTION OF THEOPHYLLINE

Add 30 cc. of $CHCl_3$-ethyl alcohol (3+1) mixture to the main aqueous solution and shake 1–2 minutes. Allow to settle and transfer the lower layer into the second separator containing the 10 cc. of acidified water that was used to wash the CCl_4-ether solvent. Shake well, allow to settle, and filter the solvent through a filter paper saturated with the solvent, at first into a large (250 cc.) beaker. Repeat with six

successive portions of 20 cc. $CHCl_3$-ethyl alcohol solvent until the alkaloid is removed completely (more extractions may be necessary), washing each portion with the acidified water in the second separator. Dissolve any theophylline remaining on the rim of the filter paper and funnel with fresh solvent. Evaporate the combined solvent on a steam bath with the aid of a current of air to about 20 cc., transfer quantitatively with the aid of solvent to a weighed 50 cc. beaker, and evaporate to dryness. Treat the residue with 2 cc. of absolute alcohol and evaporate to dryness, then add 2 cc. of absolute ether and evaporate cautiously with medium heat to avoid spattering. Dry the residue at 80° C. Weigh as anhydrous theophylline.

$$C_7H_8O_2N_4 \times 1.10 = C_7H_8O_2N_4 \cdot H_2O$$

Volumetric Method

Transfer 0.6–0.7 gram of the material into a 250 cc. beaker, and add 50 cc. of water and 10 cc. of 10% NH_4OH (2+3). Heat the mixture on the steam bath to complete solution. Add 30 cc. of 0.1 *N* $AgNO_3$ while stirring, cover with a watch-glass and continue to heat on the steam bath for 30 minutes. Filter while still warm through a Gooch crucible with the aid of suction and trap and wash the beaker and precipitate with three 10 cc. portions of water. Transfer the filtrate to a 500 cc. separator, using small portions of water to rinse the suction flask.

EXTRACTION OF SALICYLIC ACID

Add 3 cc. of HNO_3 and 50 cc. of $CHCl_3$-ether solvent (3+1). Shake well and allow to settle. Transfer the lower layer to a second separator. Repeat the extraction with two 50 cc. portions of the $CHCl_3$-ether solvent. Wash the combined extracts with two 10 cc. portions of water acidified with a few drops of the HNO_3, then wash the combined washings with 20 cc. of $CHCl_3$-ether solvent. Transfer the lower layer to the combined $CHCl_3$-ether extracts in the separator, and determine salicylic acid by shaking with three 20 cc. portions of 2 per cent NaOH solution and two 20 cc. portions of water. Combine alkaline extract and washings in a 200 cc. volumetric flask and make to mark. Proceed as directed in *Methods of Analysis, A.O.A.C.*, 1935, 551, **27**, beginning with "Transfer an aliquot . . . ".

DETERMINATION OF THEOPHYLLINE

Transfer the main acidified aqueous solution and combined acidified wash water to a 500 cc. Erlenmeyer (or if preferred the titration may be made in the separator from which the salicylic acid has been removed), add 5 cc. of the HNO_3, and 3 cc. of ferric ammonium sulfate T.S., and titrate the excess $AgNO_3$ with 0.1 *N* NH_4CNS.

1 cc. of 0.1 *N* $AgNO_3$ = 0.01801 gram of anhydrous theophylline.

$$C_7H_8O_2N_4 \times 1.10 = C_7H_8O_2N_4 \cdot H_2O$$

THEOPHYLLINE-SODIO SALICYLATE IN MIXTURE WITH STARCH AND LACTOSE

Method 1

Transfer a sufficient sample to contain 2.5–3.0 grams of anhydrous theophylline into a 100 cc. volumetric flask and make to mark with water. Shake well, allow to settle, and filter through a dry filter. Pipet a 10 cc. aliquot of the clear filtrate into a separator. Add 50 cc. of CCl_4-ether mixture (9+1) and sufficient HCl T.S. dropwise to insure acidity to litmus. Proceed as directed in Method 1 under "Extraction of Salicylic Acid" beginning with "Then add 1 cc. more of the acid . . . ".

Method 2

Transfer by means of a pipet a 10 cc. portion of the filtrate, obtained by Method 1, into a separator; add 30 cc. of $CHCl_3$-ethyl alcohol (3+1), sufficient HCl

TABLE 2.—*Results obtained on Sample 1 with proposed methods*

COLLABORATOR	ALTERNATIVE METHODS USED							
	GRAVIMETRIC METHOD			VOLUMETRIC METHOD				
	ANHYDROUS THEOPHYLLINE	THEOPHYLLINE · H_2O RECOVERED*	AVERAGE RECOVERED	ANHYDROUS THEOPHYLLINE	THEOPHYLLINE · H_2O RECOVERED*	AVERAGE RECOVERED	SODIUM SALICYLATE	SODIUM SALICYLATE RECOVERED
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
K. L. Milstead	39.29	99.36	99.55	39.09	98.85		56.16	98.5
	39.44	99.74		39.73	100.4	99.62	55.99	98.28
H. G. Underwood	39.31	99.40		39.80	100.6		57.64	101.17
	39.73	100.40		39.76	100.5		57.64	101.17
			99.9			100.55	57.64	101.17
							56.47	99.1
L. E. Warren	39.84	100.74		39.39	99.60		56.84	99.47
	40.10	101.03	100.26	39.36	99.54	99.57	56.74	99.29
							56.46	98.80
G. M. Johnson	39.31	99.4		40.09	101.3			
	39.83	100.7	100.05	40.20	101.66	101.48		
Associate Referee	39.34	99.5		39.20	99.13		56.73	99.57
	39.30	99.38	99.44	39.06	98.8	98.86	56.5	99.17
				39.10	98.9			
				39.0	98.62			

* Corrected for partial loss of water of crystallization in the theophylline U S P. used. % H_2O found =7.75%. Theory for H_2O content in theophylline =9.09%.

(1+3) until acid to litmus, then 1 cc. in excess. Shake for 1–2 minutes and allow to settle. Transfer the lower layer into a second separator and repeat the extraction with six successive portions of 20 cc. $CHCl_3$-ethyl alcohol solvent. Wash the combined extracts with two 10 cc. portions of water, then wash the combined washings with 20 cc. of $CHCl_3$-ethyl alcohol solvent. Transfer the lower layer to the combined $CHCl_3$-ethyl alcohol extracts in the separator and filter the solvent through a filter paper into a 250 cc. beaker. Evaporate the bulk of the combined solvent on the steam bath with the aid of a current of air and complete by spontaneous evaporation. Add 40 cc. of water and 10 cc. of ammonia T.S. Proceed as directed in Method 2, beginning with "Heat the mixture . . . ".

TABLE 3.—*Results obtained on Sample 2 (alternative methods used)*

COLLABORATOR	GRAVIMETRIC		VOLUMETRIC		
	ANHYDROUS THEOPHYLLINE	RECOVERY*	ANHYDROUS THEOPHYLLINE	RECOVERY*	SODIUM SALICYLATE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
L. E. Warren	30.18 29.33	99.34 96.54	30.82 30.41	101.4 100.1	
H. G. Underwood	27.64 27.4	91.0 90.2	28.10 28.01	92.5 92.2	
Associate Referee	30.11 29.90	99.11 98.4			34.90 34.73
K. L. Milstead	26.93	88.64	26.76	88.07	36.0 35.77

* Based on the average of 42.54% anhydrous theophylline found in the commercial product "Theophylline-Sodio Salicylate."

DISCUSSION

After considerable experimental study, carbon tetrachloride-ether was found to be the ideal solvent as it enabled the quantitative separation of the salicylate without removing theophylline also, since theophylline is soluble in carbon tetrachloride only to the extent of 1 part in about 30,000 and in ether of 1 part in about 3,000.

COMMENTS BY COLLABORATORS

K. L. Milstead.—The salicylates may be determined more rapidly by the following procedure: Wash the combined solvents obtained in either Method 1 or 2 with 3–10 cc. portions of water, then wash the combined washings with a 30 cc. portion of the corresponding solvent. Transfer the lower layer to the combined extracts and filter the solvent through a filter paper into a 250 cc. beaker. Evaporate the bulk of the combined solvent to about 5 cc. at a temperature not exceeding 40° C., add 25 cc. of diluted alcohol that has been previously neutralized with 0.1 *N* NaOH, using phenolphthalein T.S. as the indicator, and titrate with 0.1 *N* NaOH until the pink color is restored. Each cc. of 0.1 *N* NaOH is equivalent to 0.016 gram of sodium salicylate.

L. E. Warren.—In removing salicylic acid and theophylline from the lactose, I would suggest the use of some form of automatic extraction apparatus. I have

found that the Murray-Palkin-Watkins extractor of the type for liquids heavier than water works well for this separation, when the chloroform-ethyl alcohol mixture (3+1) is used.

In Method 2 if the extraction of salicylic acid be made in a 500 cc. separator, the excess of $AgNO_3$ may be titrated directly in this container.

SUMMARY

The gravimetric and volumetric methods for the determination of the pure product theophylline sodium salicylate give concordant results.

The volumetric method is more rapid, but in the opinion of the Associate Referee the gravimetric method is useful as an alternative method.

Results obtained in the assay of Sample 2, which contained the excipients, show that further study of the methods is necessary.

RECOMMENDATIONS[1]

It is recommended—

(1) That the gravimetric and volumetric methods for the pure product theophylline sodium salicylate be tentatively adopted.

(2) That the method for determining the product in the presence of excipients be further studied.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 21, 67 (1938)

THIRD DAY

WEDNESDAY—MORNING SESSION

REPORT ON FEEDING STUFFS

By L. S. WALKER (Agricultural Experiment Station, Burlington, Vt.), *Referee*

During the past year the following subjects have come to the attention of the Referee on Feeding Stuffs. These may merit consideration.

First, in regard to the determination of ash in feeds, J. L. St. John, Pullman, Wash., and J. W. Clulow, Seattle, Wash., state that in using the official method for the determination of ash in feeds containing calcium carbonate, they have experienced difficulty in obtaining good checks. The individual interpretations of what constitutes "dull red heat" and "until free from carbon" seem to be the cause of the trouble.

The second subject is in regard to the determination of manganese in poultry feeds. This is the result of recent discoveries of the effect of manganese on "slip tendon" in chickens. Apparently a different procedure from that used on fertilizer is required for this determination on feeds.

It would seem that both of these subjects should be studied, and therefore it is recommended that associate referees be appointed for this purpose.

The method for the determination of fluorine is being studied on both foods and feeds by the same associate referee. This is a duplication of work. Therefore it is recommended that fluorine in feeds be discontinued until a method applicable to foods is adopted.

REPORT ON FLUORINE IN FEEDING STUFFS

By DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work of the Associate Referee this year was confined to the analysis of a few raw materials used in feeding stuffs. No collaborative work was done, because the Associate Referee considers that satisfactory methods for sample preparation should be worked out first. Much of this work, incidentally, will be common to both foods and feeding stuffs.

The following materials were analyzed during the year: Cottonseed meal, cottonseed hulls, oats, corn, and corn germ. With these materials the chief analytical problem is one of obtaining a rapid and complete contact between the sample and the fluorine fixative during the ashing necessary for destruction of organic matter. At present this step is entirely

too time-consuming. A lack of agreement was also found between fluorine results on the same sample treated by different methods of preparation. This is shown in Table 1, giving analytical results, as well as the limits between which they varied on the same sample. Since both the materials

TABLE 1.—*Fluorine content of food materials*

MATERIAL	FLUORINE FOUND (P.P.M.)
Cottonseed meal	20–31
Cottonseed hulls	12–14
Corn	1– 2
Corn germ	8–11
Oats	3– 4.5

showing the highest fluorine also contained a fairly high percentage of fat, extractions were made and fluorine was determined separately on the extract and the residue. The results follow:

MATERIAL	EXTRACTED WITH—	AMOUNT OF EXTRACT		FLUORINE CONTENT (P.P.M.)		
				WHOLE SAMPLE	EXTRACT	RESIDUE
		grams	*per cent*			
Cottonseed meal	Chloroform	3.0	6.0	20.3	18.3	23.0
Corn germ	Ether	2.6	52 0	11.0	4.6	18.5

The chloroform extraction of cottonseed meal was carried out at ordinary temperature, and the ether extraction of corn germ in a modified Soxhlet apparatus.

It is recommended[1] that the work on fluorine in feeding stuffs be temporarily discontinued, pending developments on methods for preparation of sample for the determination of fluorine in foods.

REPORT ON STOCK FEED ADULTERATION

By P. B. CURTIS (Department of Agricultural Chemistry, Purdue University, Lafayette, Ind.), *Associate Referee*

During the past year the Associate Referee on Stock Feed Adulteration confined his work to a study of methods for the detection of adulteration of condensed milk products.

In order to enhance the value of condensed milk products for feeding purposes, a few feed manufacturers are incorporating various feed and mineral ingredients in their condensed buttermilks and skimmed milks.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 60 (1938).

Some of these ingredients are cod liver oil, corn germ oil, wheat germ oil, carotene, tomato juice, molasses, sulfur, potassium iodide, tallow, and cereal grasses. The addition of these ingredients can not be considered adulteration as long as the correct ingredients are declared. However, if an undeclared ingredient, such as a low-grade flour or a starch, is used to increase the total solid content of a partially condensed milk product, such a procedure would constitute adulteration.

Fortunately, starch or starchy materials can be readily detected qualitatively in condensed milk products. The following method, which has been used by the Associate Referee in feed control work, has proved to be a rapid reliable method:

Thoroughly mix approximately 2 grams of the condensed milk product with 100 cc. of distilled water, and heat the mixture to boiling. Place a portion of the mixture on a spot plate and add a drop of iodine solution. If starch is present, an intense blue color will be produced.

This qualitative test is quite sensitive for starch and can be used to detect its presence in minute quantities. A sample containing a few tenths of one per cent of starch can be readily detected. In order to secure maximum color results the proper dilution of the condensed milk product is quite important. Concentrated solutions tend to mask the blue color when small quantities of starch are present.

Because of its simplicity and the short time required to carry out this test for starch, it may be desirable to have the method studied by collaborators.

RECOMMENDATIONS[1]

It is recommended—

(1) That further study be made of methods for the detection of adulteration of condensed milk products.

(2) That a study be made of methods for the detection of adulteration of cod liver oil.

REPORT ON MINERAL MIXED FEEDS

By Alfred T. Perkins, *Associate Referee*, and J. F. Merrill (Department of Chemistry, Kansas State College, Manhattan, Kan.)

For the current year the major part of the Associate Referee's work has been devoted to iodine methods. The reports for 1935 and 1936 by Halvorson, *This Journal*, **18**, 335 (1935) and **19**, 581 (1936), were studied in detail and it was decided to study the Elmslie-Caldwell method. An accurately prepared sample of low-grade limestone was treated with 0.03 per cent of iodine as potassium iodide. This sample was used as a standard

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 60 (1938).

throughout the preliminary work. Details of the Elmslie-Caldwell method are given in *This Journal*, **18**, 338 (1935).

The first point tested was the acidity of the solution before being treated with bromine water. In some cases methyl red and in other cases methyl orange were used as an indicator. Varying amounts of phosphoric acid were added. The results obtained are given in Table 1.

TABLE 1.—*Effect of acidity on iodine titration*

CONC. H_3PO_4	INDICATOR	INDICATOR CHANGE	IODINE TITRATED	REMARKS
cc.			*per cent*	
5	M. R.	None	None	Very unstable end point
6	M. R.	Very slight yellow	5	Very unstable end point
7	M. R.	Slight yellow	5	Very unstable end point
9	M. R.	Orange yellow	15	Very unstable end point
11	M. R.	Slight red	95	Unstable end point
13	M. R.	Full red	100	Satisfactory titration
11	M. O.	None	95	Unstable end point
12	M. O.	Slight yellow	100	Satisfactory titration
13	M. O.	Pink	100	Satisfactory titration
15	M. O.	Full red	100	Satisfactory titration
17	M. O.	Full red	100	Satisfactory titration
20	M. O.	Full red	100	Satisfactory titration

From the results (Table 1) it was assumed that satisfactory oxidation of the iodine to iodate would not occur if the acidity of the solution was too low. The least amount of phosphoric acid (H_3PO_4) that could be used with safety was that which would produce the full color change of methyl orange. An excess of acid apparently causes no trouble.

The next point to be tested was the volume of solution on addition of the concentrated acid. The Elmslie-Caldwell method calls for a volume of 300 cc. Volumes of 100, 150, 300, 450, and 600 cc. were tried. In every case the solution was kept cool on the addition of the acid, and in every case 100 per cent of the iodine was recovered. However, in later work, when care was not taken to cool the solutions, it was apparent that in the lesser volumes a slight loss of iodine occurred on the addition of the phosphoric acid (H_3PO_4).

The time of boiling was tested, and optimum results were obtained with a 5 minute boiling period. Shorter or appreciably longer boiling periods gave low results. However, the apparent loss of iodine was not significant or beyond experimental error when the solution was boiled 2–10 minutes.

The next point tried was the recovery of varying amounts of iodine. The Elmslie-Caldwell method specifies a sample containing 3–4 mg. of iodine, but it was tested for amounts of 1–25 mg. In every case the iodine recovered ran over 99.5 per cent of the theoretical amount, which indi-

cates that the method is reliable for amounts of iodine far beyond those stated. However, a sample containing 3 or 4 mg. of iodine gives a very convenient titration volume, and in most cases is contained in a satisfactory amount of sample.

It has been suggested that in washing the disintegrated fusion some iodine might remain behind. Possibility of ionic adsorption as well as other factors indicated that a wash solution of normal sodium chloride might be substituted for the distilled water. In both cases, however, identical results of 100 per cent iodine recovery were obtained.

The temperature of fusion was also tested. No iodine was lost when the nickel crucible was heated to incipient redness, the crucible showing only the faintest tinge of red while in the muffle in a darkened room. Under some conditions low results were obtained when the crucible did not reach the temperature or maintain it for 15 minutes. Under other conditions some iodine was lost when the heating was prolonged or was too high. Other tests included partial neutralization of the fused mass before filtration, the purpose being to prevent occlusion at this time and thus improve checkability. Later results obtained by collaboration disproved this possibility.

Likewise, additions of 1 per cent copper sulfate, potassium permanganate, and ferric chloride were made to the sample. The basic Elmslie-Caldwell method apparently takes care of such elements in small amounts as they did not affect the results obtained.

It also seemed possible that some revisions would improve the checks obtained by several laboratories. Consequently a revised method was formulated, and samples were sent to certain collaborators. The revised method follows:

REVISED METHOD

Place a sample that contains about 3–4 mg. (1) of iodine into a 100 cc. or larger nickel crucible. Add approximately 5 grams of Na_2CO_3 and moisten completely with 95% ethyl alcohol; add 5 cc. of a saturated NaOH solution and mix thoroughly (2). Dry at about 100° C. so that there will be no spattering upon subsequent heating (30 minutes is usually sufficient).

Place the crucible and contents in a muffle furnace previously heated to a very faint tinge of redness and keep in the muffle for 15 minutes (3). Cool, add 25 cc. of distilled water, cover the dish with a watch-glass, and boil gently for 10 minutes. Transfer the contents with water to a 400 cc. beaker (4). Dilute to about 200 cc., cool (room temperature), add about 0.5 cc. methyl red solution (1%) and H_3PO_4 (85%), slowly stirring until the initial color change is maintained for 5 minutes. Filter, wash thoroughly with hot water, cool the filtrate, and add H_3PO_4 (85%) dropwise to the full color change of the M.R. (5) and 7 cc. in excess.

Add an excess of bromine water and boil the solution gently until colorless, and then 5 minutes longer. Add a few crystals of salicylic acid and cool the solution to about 20° C. Add 1 cc. of 85% H_3PO_4 and about 0.5 gram of KI and titrate the iodine with 0.005 N $Na_2S_2O_3$ in the usual way, using starch solution as indicator.

Standardize the $Na_2S_2O_3$ solution by measuring into a beaker exactly 25 cc. of a solution containing 0.1308 gram of KI per liter, adding 300 cc. of water and 5 grams

of Na_2CO_3, neutralizing, and proceeding as above. (It is advisable to standardize on the same day the determinations are conducted.)

NOTES

(1) The method gives satisfactory results with amounts of iodine not too far outside the 3–4 mg. limits, but this amount is considered optimum.

(2) When adding the NaOH solution, avoid water, rinse stirring rod with alcohol.

(3) Minimum heat to char the sample is desirable. The alkaline base will help hold the iodine. Ignition of the sample appears to be necessary only to carbonize any soluble organic material that would be oxidized by bromine water if not so heated (500° C. is a satisfactory temperature).

(4) In breaking up the ignited mass the flat end of a porcelain pestle is convenient. The H_3PO_4, if diluted, may be used to help clear the crucible if it is kept cool.

(5) The best indicator for the titration is methyl orange, but the optimum pH value for the filtration is above the range of M.O. Methyl red is therefore used, and the 7 cc. excess of H_3PO_4 is later used to reach the acidity of the M.O. change.

Those collaborating were:

1. E. R. Tobey and Millard G. Moore, Maine Agr. Experiment Station.
2. Oscar I. Struve, Eastern States Cooperative Milling Corp.
3. C. Perry Coleman, Agricultural Dept., State of Florida.
4. M. Elmer Christensen, State Chemist, Utah.
5. E. M. Bailey and W. T. Mathis, Connecticut Agr. Experiment Station.
6. W. P. Elmslie and W. R. Bunting, Moorman Manufacturing Co.
7. H. B. McDonnell, University of Maryland.
8. H. R. Kraybill, P. B. Curtis, and L. J. Swift, Purdue University Experiment Station.
9. L. W. Davis, National Oil Products Co.

Several others began collaboration, but owing to certain difficulties did not report. Two samples were sent out, and the results are given in Table 2.

TABLE 2.—*Results by the revised method*

COLLABORATOR	SAMPLE A	SAMPLE B
	per cent	*per cent*
1	0.0252	0.0218
2	0.0265	0.0249
3	0.0306	0.0288
4*	0.0235	0.0237
5	0.0282	0.0257
6†	0.0230	0.0286
7	0.0220	0.0240
8‡	0.0221	0.0253
9§	0.0237	0.0235

* 10 gram sample—a 5 gram sample gave lower results.

† Reported somewhat lower results by Elmslie-Caldwell method but noted a loss of iodine from Sample A.

‡ Reported 20% and 8% lower results by Knapheide-Lamb method.

§ Speaks of free sulfur interfering in Sample B.

A. Theoretically contained 0.030 per cent iodine as KI and impurities as Fe, Cu, Mn, Cl, etc. It is known that these probably caused some loss of iodine, as free iodine was detected in stored samples.

B. Theoretically contained 0.025 per cent iodine as KI plus a trace of iodine in agar.

One of the collaborators suggested that the fused mass might be left overnight in water for disintegration.

The results obtained by the revised method (Table 2) seem to be no better than those reported for the Elmslie-Caldwell method in 1935. It is the opinion of the Associate Referee that the Elmslie-Caldwell method is as accurate as any short method can be made and that it compares very favorably with longer, more detailed methods. No modification of the Elmslie-Caldwell method has yielded any greater degree of checkability among several laboratories than the original method. Certain errors of standardization, titration, weighing, etc., are inherent in any method, and improved checkability will depend on more precise technic than is common or might rightfully be expected in the average laboratory. The results reported in 1935 and in this report show that the Elmslie-Caldwell method or its revision will determine the iodine in a 10-gram sample accurately in grams to the fourth decimal place. The replies to several attempts to get biological chemists to state the accuracy necessary in a method for determining iodine indicate that 0.01 per cent is sufficient, and the Elmslie-Caldwell method comes within this range. To date the Elmslie-Caldwell method has been proved under diverse conditions, and has yielded accurate results in the presence of all factors that would likely interfere with its accuracy. It is recommended[1] that the Elmslie-Caldwell method published in *This Journal*, **18**, 338 (1935), be adopted as a tentative method.

REPORT ON LACTOSE IN MIXED FEED

By D. A. Magraw (American Dry Milk Institute, Chicago, Ill.), *Associate Referee*

A method for the determination of lactose in mixed feed was published recently by Magraw and Sievert.[2] The method was later improved, and the improved method was published by Magraw, Copeland, and Sievert in *This Journal*, **19**, 605 (1936).

COLLABORATIVE STUDIES

The method came to the attention of the A.O.A.C. and was recommended for collaborative study during 1937.

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, **21**, 60 (1938).
[2] *Ind. Eng. Chem. Anal. Ed.*, **7**, 106 (1935).

TABLE 1.—*Formulas of samples**

	1	2	3	4	5	6	7	8	9	10	11
Corn	34	26	46	35	30	22	25	32.25			
Bran	10	10	5	10	15	15	12.5	10			
Standard middlings	10	20	15	20	25	20					
Flour middlings	10									47	
Fine ground oats	5	20	10	5		15	34.5				
Alfalfa	5	5	2.5	5	5	5			20		10
Meat scraps	5	2.5	5	5		20					
Soybean oil meal	5	5	5	5	2.5				12.5	10	32.5
Fish meal	2.5	2.5	2.5	2.5				3			
Calcium carbonate	2		2	2	3.5	2	1	.5		1	
C.L.O.	1	1	1	1				.25		.5	
Salt	.5	1	1	1	1	1	1	.5		.5	
D.S.M.	10	5.0	2.5	1	0.5			20	5	10	
Mineral		2							5		7.5
Peanut meal			2.5	2.5	2.5						
Corn gluten feed				5							
Tankage					10				45	10	30
Cottonseed oil meal					2.5						15
Gluten feed					2.5						
Linseed oil meal							12.5	5	12.5	10	5
Blood meal							12.5				
Steamed bone meal							1	.5		1	
Rolled oats								28			
Com. oil cake meal										10	

* Feed sample No. 1—Chick Starter
2—Growing mash
3—All mash laying mash
4—Competitive milk mash
5—Cheap chick mash
6—Poultry mash
7—Dry calf meal
8—Dry calf starter
9—Hog concentrate
10—Pig meal
11—Protein concentrate

TABLE 2.—*Collaborative results*

SAMPLE NO.	1	2	3	4	5	6	7	8	9	10	11
Actual Lactose (%)	5	2.5	1.25	.50	.25	0	0	10.0	2.50	5.0	0
Collaborator											
1	4.75	2.26	1.21	.23	.06	0	0	9 55	2.08	4.46	0
2	4.85	2.46	1.17	.49	.30	0	0	9.70		4.98	0
3	4.85	2.45	.98	.35	.08	0	0	9.51	2.07	5.08	0
4	4.97	2.57	1.16	1.03	.46	.08	.04	10.11	2.61	5.37	0
5	4.90	2.72	1.22	.63	.26	0	.04	9.25	2.06	5.06	0
6	5.10	2.89	1.34	.83	.37	0	0	9.81	2.14	5.10	0
7	5.29	3.04	1.65	1.15	.50	.11	.03	9.83	1.20	4.92	0
8	4.48	2.21	.86	.30	.03	0	0	8.85	.81	4.10	0
9	4.73	2.50	1.19	.41		0					
Maximum	5.29	3.04	1.65	1.15	.50	.11	.04	10.11	2.61	5.37	0
Minimum	4.48	2.21	.86	.23	.03	0	0	8.85	.81	4.10	0
Average	4.88	2.57	1.19	.60	.25	.02	.01	9.70	1.85	4.88	0
Av. Deviation	±.17	±.22	±.14	±.27	±.15			±.29	±.48	±.30	

Eleven samples of feed, containing the more common feed ingredients used in commercial feed mixes, were hand-mixed in the laboratory. The dry skim milk used contained 50.7 per cent lactose as determined by the official Munson and Walker method. It was weighed directly into each individual sample that was sent out so that no variations could occur between samples due to poor mixing.

Table 1 shows the formulas for the eleven samples.

The samples were then sent to the collaborators with detailed directions, an elaboration of the procedure previously published.

RESULTS

The results of the analyses made by the nine collaborators are presented in Table 2. In most cases the results are the averages of several determinations.

COLLABORATORS

1. A. H. Johnson, Baltimore, Md.
2. A. M. Besemer, San Francisco, Calif.
3. A. T. Perkins, Manhattan, Kan.
4. O. I. Struve, Buffalo, N. Y.
5. H. R. Kraybill, Lafayette, Ind.
6. L. E. Copeland, Chicago, Ill.
7. W. B. Griem, Madison, Wis.
8. G. H. Marsh, Montgomery, Ala.
9. H. A. Halvorson, St. Paul, Minn.

DISCUSSION

If the average on the results is taken into consideration, as well as the average deviation, the method seems to be quite satisfactory, with the exception of Sample 9, which will be discussed later. If the maximum and minimum results are considered, they are entirely too wide. There seem to be several factors that contribute to these variations in the results. The factors evident from the data received from the different collaborators are the following: (1) Fermentation temperature, (2) wide variations of Cu_2O obtained on feeds containing no lactose, (3) indications of peanut meal being an interfering substance, and (4) difficulty of clarifying of feeds containing combinations, e.g., Sample 9.

Experience in this Laboratory indicates that the temperature of fermentation is even more important than was evident in previous experiments. At the time this work was done it was very warm in Chicago and it was difficult to maintain a low temperature during the fermentation. Each time the temperature reached 33° C. or above the results were erratic and as a rule were low. After the weather had cooled off and the temperatures of fermentation could again be held at a maximum of 29° C. and a minimum of 27° C. dependable and duplicatable results were obtained. It was also noted that there was considerable variation in the temperatures used by the different collaborators.

The results and also the comments made by the collaborators show that there is considerable variation in the quantities of cuprous oxide obtained on feeds containing no lactose, which should be subtracted from the results on samples containing lactose as a correction. Many of the collaborators obtained no cuprous oxide on these blank samples, and in these cases the majority of their results were low to the extent of approximately the amount of the blank.

Results on Sample 8 probably show wide variation because it was a feed of coarse texture. Evidently all collaborators did not grind this sample. Several collaborators called attention to this point, which may well be included in the analytical procedure, although it may also be argued that suitable preparation of sample is always necessary before any analytical procedure is followed.

Samples 3, 4, and 5, which gave erratic results, contained peanut meal,

TABLE 3.—*Precipitation with alcohol*
(Fermentation temperature 83°–84° F. No blank taken off)

SAMPLE NO.	Cu_2O	LACTOSE	LACTOSE DETERMINED	LACTOSE ADDED
	mg.	*mg.*	*per cent*	*per cent*
1	164.8	111.0	4.96	5 0
	170.3	114.9	5.13	
2	88.89	58.9	2.63	2 50
	89.76	59.5	2.66	
3	44.99	28.9	1.29	1.25
	44.11	28.3	1.26	
4	21.2	13.3	.60	.50
	22.05	13.8	.62	
	23.6	14.7	.66	
	23.6	14.7	.66	
5	15.54	9.7	.43	.25
	15.07	9.4	.42	
6	—	—	—	—
	—	—	—	
8	320.0	218.7	9.76	10.0
	321.5	219.7	9.81	
9	81.2	53.7	2.40	2.50
	81.9	54.8	2.45	
10	158.6	106.9	4.78	5.00
	151.2	101.7	4.54	

which had not been previously noted as an interfering substance. Sample 9 also seemed to give results similar to Nos. 3, 4 and 5, except that Sample 9 always gave low results, while Samples 3, 4, and 5 gave both high and low results. Apparently the substance causing trouble in Sample 9 was the high percentage of tankage, which was of a very low grade.

A number of comments received stated that considerable difficulty was encountered in clarifying these samples, especially Nos. 9 and 5. The same difficulties were encountered in the writer's laboratory, and an effort was made to overcome them by the addition of an equal volume of 95 per cent ethyl alcohol to the 150 cc. of filtrate following the water extraction of the feed. The precipitate was centrifuged off, and 250 cc. of the filtrate was evaporated down until no odor of alcohol was perceptible, and then transferred to a 200 cc. volumetric flask, the volume being held between 100 and 125 cc. The regular procedure was then followed with the exception of making the appropriate changes in final calculations to allow for the change in volumes. The results on these samples indicated that no blank need be taken off for correction and the percentage of lactose was much nearer the actual quantity of lactose added. No difficulties were experienced with Samples 3, 4, or 9.

CONCLUSIONS

The results of the nine collaborators on the eleven samples submitted to them show promising possibilities, but they indicate that further study is needed.

It is recommended[1] that the study be continued for another year and that consideration be given to—

1. Working out a more satisfactory blank.
2. Fermentation temperature—maximum and minimum.
3. The possibility of using alcohol to eliminate interference due to peanut meal and low-grade tankage.

REPORT OF COMMITTEE ON MOISTURE

By H. A. Halvorson (Department of Agriculture, St. Paul, Minn.), *Chairman;* P. B. Curtis and P. A. Clifford

The purpose and working plans of the Committee on Moisture are given in Lepper's report as Chairman of the Committee on Recommendations of Referees, *This Journal,* **20,** 45 (1937), and in the Associate Referee's report on moisture, *Ibid.,* 435. With these broad objectives the Committee has necessarily limited its work this year to such unification of procedures as may be accomplished without extensive collabora-

[1] For report of Subcommittee A and action by the Association, see *This Journal,* **21,** 60 (1938).

tive tests. It was decided to attempt correlation of the vacuum oven methods for grain and stock feeds, *Methods of Analysis, A.O.A.C.*, 1935, 335, **2**, and for dried milk and malted milk, *Ibid.*, 282, **68**, with the vacuum oven method for wheat flour, *Ibid.*, 206, **2** and **3**, also the electric air-oven method for feeding stuffs, *Ibid.*, 336, **7**, with a similar method for wheat flour, *Ibid.*, 207, **4**. In many respects specifications of the individual procedures within the two types of methods are identical, and there is also similarity in composition of the three classes of products.

To obtain authoritative opinions with reference to changes that would bring the methods into unison, inquiries were made of the General Referees on Cereal Products and Dairy Products as follows:

1. Is it not possible to unify the three vacuum oven methods so that the same equipment, utensils, and technic can be used for the three classes of materials? The consensus of opinion is "yes," since changes recommended here affect only factors that have negligible influence on results. Slight variations in size of dishes are noted, and apparently no objection can be raised to a recommendation that their measurements in all methods shall read the same. The directions for wheat flour and for dried milk and malted milk provide that drying of samples shall be done with covers fitting loosely on dishes, while for feeding stuffs no such instructions are given. There seems to be no reason why the latter method cannot be revised to agree with the other two. The directions for flour and stock feeds give drying temperatures of 98–100° and 95–100° C., respectively, while the dried milk vacuum oven method (still tentative) specifies the uncertain "temperature of boiling H_2O." The Committee considers that there would be no objection to specifying a drying temperature of 98–100° C. in all three methods. Since only slight variations exist in size of samples, uniformity in this respect can be secured by directing use of "about 2 grams." As no directions for admitting dry air to the vacuum chamber at the conclusion of the drying period is provided in the feeding stuffs method, it would be well to include this detail as in the case of the others. Directions for admitting a slow current of dried air during drying should be omitted in the dried milk method as it is superfluous.

2. In view of the fact that methods for feeding stuffs and dried milks allow pressures as high as 100 mm. of mercury in the vacuum chamber during drying, the second question raised was whether or not adequate reasons exist for the provision requiring pressure equivalent to 25 mm. or less of mercury in the wheat flour method. The report of Grattan, *This Journal*, **17**, 178 (1934), shows surprising lack of uniformity of pressure employed in the vacuum chamber of ovens in 47 laboratories canvassed. It is also the opinion of the Committee and of several qualified chemists interviewed that the great majority of both official and commercial feed and flour laboratories maintain pressure between 50 and 100 mm. of mercury rather than under 25 mm. Accordingly, it is believed that investigations should be made to determine for all methods the uniform optimum pressure that will produce results in consonance with those obtained under specifications on which present standards for moisture are based.

3. Explanation of basis for the third question stated that since estimation of moisture in biological substances is an empirical procedure and results depend upon varying conditions of time, temperature, pressure, and composition of materials aside from water content, no purpose is served by the drying-to-constant-weight provision in the three methods. The recommendation of the Committee, which is supported by concurrence of the General Referees on Cereal and Dairy Products, is

that consideration be given to omission of these statements and 5 hours of drying definitely specified.

4. The fourth item, that of reconciling the air-oven methods for flour and feeding stuffs, was raised partly as the result of a communication received from O. I. Struve of the Eastern States Farmers Exchange in which evidence was presented supporting the desirability of dropping the feeding stuffs method, which requires 2 hours' drying at 135° C., and substituting for it the wheat flour procedure, in which samples are dried 1 hour at 130° C. He states that after much collaborative work and discussion flour and feed laboratories in the vicinity of Buffalo have adopted 1 hour drying at 130° C. as being dependable for feeds and feeding materials, and also reports that it has been definitely established that the longer period at the higher temperature scorches corn gluten meal, some fish meals, molasses mixtures, and mixed feeds containing them.

Treloar and Sullivan[1] statistically compared results of the two air-oven methods with vacuum oven tests on four different samples of mill feeds. They state: "Consistency of results in replicate analyses appears to be greater by the vacuum-oven method than by the air-oven methods . . . and moisture driven off from feeds by using an air oven for 2 hours at 135° C. exceeds by about 0.3 per cent (absolute) the moisture evaporated by using an air oven for 1 hour at 130° C. or a vacuum oven for 5 hours at 98–100° C. The latter two methods give essentially the same results. One conclusion is clear—superiority in consistency of results by Method 3 (2 hours at 135° C. in air oven) is not in any way demonstrable from these investigations."

It may be recalled that moisture reports to the Association by collaborators in the past contained conclusions somewhat at variance with statements in the two foregoing paragraphs. For example, Grattan, *This Journal*, **11**, 162 (1928), reported more moisture and better agreement with results by the vacuum oven procedure in four mill feeds (representing bran, shorts, middlings, and feed flour) at the end of 2 hours' drying in an air oven at 135° C. than after 1 hour. Results after 2 hours' drying at 135° C. were also higher and in better agreement with vacuum oven results than those after a like period at 130° C. This investigator further states, *This Journal*, **14**, 153 (1931), that while molasses feeds are carmelized and floury portions of bran become a shade darker after two hours' drying at 135° C., it appears the procedure has little chemical effect on cereal feeds, meat by-products, and oil cake meals, but causes decomposition of feeds containing sugars. This report also confirms the previous conclusions that at 135° C. 2 hours' drying gives higher results than does drying for 1 hour. However, conclusions from this investigation with respect to scorching and higher moisture results after 2 hours' drying are weakened because of large samples (5 grams) used. Spencer, *This Journal*, **9**, 404 (1926), in a series of tests with flour in which samples were dried by the vacuum oven method and by a procedure like the air-oven method for flour but with drying temperatures at 125°, 130°, and 135° C., concludes that variations of 5° or less from 130° C. have little effect on results.

The foregoing differences in conclusions seem to justify a recommendation that further work be done to determine whether the air oven method for feeding stuffs should be kept official and if so, the temperature and drying period that will give results in best agreement with tests by the vacuum oven procedure. In this study the following precautions called to the attention of the Committee should be observed: Ovens used should be of such type that there is a minimum of variation in the different shelves on which the moisture dishes are placed, and the analyst should be cautioned that the drying time should be for the full period at the specified temperature, since overloading ovens with many samples causes a drop in temper-

[1] *Cereal Chem.*, **12**, 520 (1935).

ature after the dishes are placed on the shelves. Additional time should therefore be allowed for the oven to reach the proper temperature.

RECOMMENDATIONS[1]

It is recommended—

(1) That investigative work be done to determine a suitable uniform pressure for vacuum oven methods for flour, feeding stuffs, and dried milk products, in order to produce results in harmony with those obtained by procedures specified when the present moisture standards were adopted.

(2) That further studies be made of the electric air-oven methods for wheat flour, grain, stock feeds, and other products to determine their adequacy for official standing.

(3) That the work of correlating moisture methods of the Association be continued.

REPORT ON BIOLOGICAL METHODS FOR ASSAY OF VITAMIN D CARRIERS

By W. B. Griem (Wisconsin Department of Agriculture and Markets, Madison, Wis.), *Associate Referee*

In 1936 the tentative A.O.A.C. method for assay of vitamin D carriers was revised in several important details, *This Journal*, **20**, 72 (1937). The feeding period was shortened from 4 to 3 weeks, optional composite ashing of the chick tibiae of each group was included, the U. S. Pharmacopoeia Reference Cod Liver Oil was specified as the standard for comparison, and the A.O.A.C. chick unit of vitamin D was defined.

The establishment of this A.O.A.C. chick unit of vitamin D, based on the effectiveness of the vitamin with chickens, has been of definite value. It is well known that some commercial sources of vitamin D, when measured by the U.S.P. unit, do not have the same ratio of efficiency on chicks as that attributed to cod liver oil. The high potency fish liver oils now used so extensively for fortifying the natural vitamin D potency of cod liver oil and sardine oil are especially variable in efficiency.

The Associate Referee was instructed to initiate collaborative study of the revised tentative method. Instructions and a sample of blended cod liver oil were submitted to the 13 collaborators who found it possible to participate in the study. The cod liver oil sample was a mixture of ten oils that had been collected in routine inspection work in Wisconsin. The Associate Referee had reason to believe that the mixture contained over 100 A.O.A.C. chick units of vitamin D per gram. Two ounces of the U.S.P. Reference Cod Liver Oil was sent directly to each collaborator from the U. S. Pharmacopoeia Convention, Philadelphia, Pa. This oil by definition contains 95 A.O.A.C. chick units of vitamin D per gram.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **21**, 61 (1938).

The collaborators were asked to feed this oil at levels of 10/95, 15/95, 20/95, and 25/95 of one per cent, equivalent to 10, 15, 20, and 25 A.O.A.C. chick units of vitamin D per 100 grams of basal ration; to feed the submitted sample of cod liver oil at three levels, namely 15/100, 15/125, and 15/150 of one per cent; and to include a negative control. Corn oil was to be added so as to make total oil additions of 1 per cent in all cases. The collaborators were also asked to average the tibia ash results of the bones of each group and also to determine the composite ash values made by unit ashing of the opposite tibiae. Interpretation of results is not specified in the method, but each collaborator was asked to interpret his own results and indicate the vitamin D potency of the submitted oil in A.O.A.C. units per gram.

COLLABORATIVE RESULTS AND INTERPRETATION OF POTENCY

The Associate Referee received reports from the following collaborators:

1. J. W. Clulow, Albers Bros. Milling Co., Seattle, Wash.
2. T. D. Sanford, F. E. Booth Co., Inc., Berkeley, Calif.
3. M. D. Thaxter, California Packing Corp., San Francisco, Calif.
4. R. F. Mann, Health Products Corp., Newark, N. J.
5. H. A. Halvorson and J. W. Perish, Minnesota Dept. of Agriculture, Dairy & Food, St. Paul, Minn.
6. G. Zinzalian, Napthole, Inc., Boonton, N. J.
7. F. D. Baird, National Oil Products Company, Harrison, N. J.
8. H. C. Schafer, Ralston Purina Company, St. Louis, Mo.
9. F. L. Gunderson and E. G. Gustavson, Quaker Oats Company, Chicago, Ill.
10. W. B. Griem.
11. R. B. Hubbell and E. M. Bailey, Connecticut Agr. Exp. Station, New Haven, Conn.
12. L. E. Bopst, University of Maryland, College Park, Md.
13. A. Black, E. R. Squibb & Sons, New Brunswick, N. J.

Table 1 shows the moisture and fat-free chick tibia ash averages from individual bone ashing and the opposite composite ashing for the eight groups of birds. One of the collaborators reported only the composite ash percentages. The potency of the collaborative cod liver oil sample, shown in the table, was interpreted by each collaborator from his own data.

No uniform method of interpretation was followed. In some cases the potency was determined by interpolation from the U.S.P. Reference Oil response curve. In some instances only one of the three ash averages produced by the different levels of the submitted oil was used in this determination of potency. In other instances the three potencies so determined were averaged. In one case the interpolated potencies from the two levels in best agreement were averaged. One collaborator used a method in which total intake of ration was included in the calculation. Another collaborator reported three values calculated from the Reference Oil ash average in closest agreement with each. Some collaborators used

TABLE 1.—*Chick tibia ash averages from individual bone ashing and opposite composite ashing. U.S.P. reference cod liver oil at four levels, collaborative cod liver oil sample at three levels and negative control. Potency in A.O.A.C. chick units per gram interpreted by collaborator*

COLL. NO.	NEGATIVE CONTROL		10/95 U.S.P. REF. C.L.O.		15/95 U.S.P. REF. C.L.O.		20/95 U.S.P. REF. C.L.O.		25/95 U.S.P. REF. C.L.O.		15/100 COLL. C.L.O. SAMPLE		15/125 COLL. C.L.O. SAMPLE		15/150 COLL. C.L.O. SAMPLE		POTENCY INTERPRETED BY COLLABORATOR IN A.O.A.C. CHICK UNITS PER GRAM
	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	INDIV. ASH AV.	COMPOSITE ASH	
1	34.06	34.46	39.58	39.31	42.15	42.43	41.51	42.26	44.00	44.21	41.87	42.35	40.81	41.17	39.25	39.48	115
2	35.30	35.50	40.20	40.30	43.10	42.60	44.30	44.60	44.80	45.00	41.90	41.50	42.50	42.40	39.50	39.60	84 (a)
3	36.36	35.80	44.43	44.55	45.10	45.58	46.81	46.85	47.36	47.91	44.55	45.60	44.98	45.13	43.92	43.49	88 ±24 (b)
4		38.98		42.65		47.94		48.18		52.90		48.72		42.45		43.48	110
5	34.39	35.18	36.23	37.92	39.68	41.65	42.49	42.10	43.23	43.23	40.59	41.52	39.72	38.76	37.64	38.54	Min. 100, Max. 125.
6	32.15	31.13	34.86	36.29	40.16	40.33	43.27	43.61	44.93	44.03	38.58	37.55	37.61	36.13	37.94	35.88	83–86.
7	33.30	33.40	35.50	34.70	35.60	35.10	38.90	38.40	39.00	40.30	37.30	37.90	35.30	34 40	34.00	34.40	133, 83, 100 (c)
8	31.61	31.74	35.51	35.93	37.32	37.81	38.87	38.64	37.59	38.23	38.07	38.38	34.10	35.14	35.59	35.86	125–133 (d).
9	36.18	35.95	44.52	44.34	46.57	46.90	47.55	48.35	47.66	48.74	46.42	46.14	45.57	44.99	46.02	46.08	106 (e).
10	34.70	34.47	38.90	39.59	40.68	40.62	41.80	41.58	42.84	42.92	41.64	41.85	40.16	40.27	39.61	39.59	Approx. 115.
11	32.70	34.00	37.70	37.70	40 20	41.70	42.10	42.80	48.10	48.70	41.40	41.40	37.30	37.40	36.30	37.10	Less than 125.
12	35.08	34.83	44.91	45.33	45.74	46.26	46.31	46.80	46.71	46.34		45.36	43.63	43.52	43.71	43.89	About 75 more than 66 less than 83
13	29.00	29.40	38.70	37.90	38.70	39.30	42.70	42.10	42.40	42.50	38 00	39.10	35.40	36.60	34.90	36.00	90–95.

(a) From composite ashings a value of 84 units was obtained from two levels, but 121 units from the 15/125 per cent level
(b) Levels specified by Associate Referee gave responses above sensitive portion of response curve Assay of dubious value.
(c) The submitted sample contained at least 100 but less than 125 and 150 A O A.C. chick units per gram based on equivalent level comparisons with U.S.P. Reference Oil Conclusive interpretation is impossible.
(d) If other levels of the U.S.P oil are compared at lower bone ash percentages the estimated potency is found to be somewhere from 100 to 115.
(e) (Collaborators included a lower level 15/300% of the collaborative sample) It is believed that the lower level of feeding (viz , 15/300%) indicating 116 A.O.A C. units is more reliable because the curve at the latter point has sharper incline—2 hour ashing for composites.

TABLE 2.—*Calcium and phosphorus content of basal ration, average bird weights, number of birds per group*

COLLABORATORS—	1	2	3	4	5	6	7	8	9	10	11	12	13
Ca content of basal ration	.76	.76	.79	.88*	.813	.78*	.98	.896	.84*	.90*	.90	.86	.86*
P content of basal ration	.65	.63	.56	.71*	.714	.75*	.60	.743	.69*	.75*	.70	.66	.77*
Calcium/Phosphorus ratio	1.17	1.21	1.41	1.24	1.14	1.04	1.63	1.21	1.22	1.20	1.29	1.30	1.12
No. of birds negative control	14	15	15	18	16	13	15	12	13	11	16	8	17
Average wt. in g. negative control	123	133	150	127	137	143	135	112	141	104	102	119	146
No. of birds 10/95 U.S.P. Ref. C.L.O.	15	15	15	18	11	13	15	11	10	13	13	9	15
Average wt. in g. 10/95 U.S.P. Ref. C.L.O.	140	129	181	140	157	156	145	136	173	126	123	152	142
No. of birds 15/95 U.S.P. Ref. C L.O.	15	14	15	18	9	15	15	11	10	23	13	10	14
Average wt. in g. 15/95 U.S.P. Ref. C.L.O.	139	158	181	159	156	161	141	121	205	120	131	122	178
No. of birds 20/95 U.S.P. Ref. C.L.O.	15	15	15	18	13	15	15	10	11	11	13	9	15
Average wt. in g. 20/95 U.S.P. Ref. C.L.O.	134	152	174	142	161	161	144	128	192	133	145	121	178
No. of birds 25/95 U.S.P. Ref. C.L.O.	13	14	15	18	12	15	15	10	10	13	14	10	15
Average wt. in g. 25/95 U.S.P. Ref. C.L.O.	145	143	175	174	161	161	170	135	195	120	150	153	195
No. of birds 15/100 Coll. C.L.O.	14	15	15	16	11	15	15	12	11	11	15	10	15
Average wt. in g. 15/100 Coll. C.L.O.	133	138	177	145	175	161	154	148	176	125	145	150	178
No. of birds 15/125 Coll. C.L.O.	12	15	15	17	13	14	15	12	11	10	15	10	15
Average wt. in g. 15/125 Coll. C.L.O.	156	145	180	153	158	156	148	135	180	134	125	136	178
No. of birds 15/150 Coll. C.L.O.	13	14	15	12	11	14	15	11	11	13	15	10	14
Average wt. in g. 15/150 Coll. C.L.O.	143	132	171	128	167	161	148	124	167	125	132	153	170

* Analyses made in laboratory of Associate Referee.

the composite ash values, while the others used the individual ash averages in their interpretations.

When the potencies are considered along with the comments of some of the collaborators on their interpretations, the Associate Referee is led to believe that, contrary to his first impression, good agreement is evident. The agreement is more remarkable when considered in connection with actual ash averages. Collaborators obtained great differences in chick responses to vitamin D intake. For example, there was a maximum difference of 9.58 per cent in the composite ashes in the negative control groups compared with 10.63 per cent on additions of 10 A.O.A.C. units per 100 grams of basal ration and 14.67 per cent on additions of 25 A.O.A.C. units per 100 grams of basal ration. The Associate Referee is unable to explain these unusual response differences. It is obvious that some uniform method for interpretation of results should be devised.

Because of the variations in the collaborators' expressions of potency no attempt was made to arrive at the average vitamin D potency of the sample. The highest value assigned by any of the collaborators was 133 A.O.A.C. chick units per gram. The lowest value was 88 plus or minus 24 A.O.A.C. chick units per gram.

Six of the thirteen collaborators reported results in the range of 100–125 A.O.A.C. chick units per gram. If the comments made by some of the other participants are considered, it can be stated that four of the remaining seven also had some evidence that the actual potency fell within this range. On the basis of the 15/150 of 1 per cent level of the submitted sample the results of Collaborator 6 also indicate a potency of somewhat over 100 A.O.A.C. chick units per gram.

The Associate Referee believes, therefore, that a potency between 100 and 125 A.O.A.C. chick units of vitamin D per gram may be accepted for the submitted sample. More experience and better agreement on details of procedure and interpretation of results should effect even greater uniformity.

RATION AND CHICK WEIGHTS

Table 2 shows the calcium and phosphorus content of the basal rachitogenic ration together with the calcium-phosphorus ratio, average chick weights, and the number of birds per group.

The calcium content of the rations varied from .76 to .98 of 1 per cent; the phosphorus content varied from .56 to .77 of 1 per cent; and the calcium-phosphorus ratio varied from 1.04 to 1.63, with 9 of the ratios falling between 1.14 and 1.30. It is interesting to note that Collaborator 7, whose ration was found to have the widest ratio, that is 1.63, obtained, on the basis of the composite ashing, the lowest responses to calcification of the chick tibiae in six of the eight groups. Collaborator 6, whose ration had the narrowest calcium-phosphorus ratio, that is 1.04, obtained re-

TABLE 3.—*Differences between composite ash of chick tibiae and the average of the individual ashes of the opposite tibiae. Numbers represent composite ash minus average of individual tibiae*

COLL. NO.	NEGATIVE CONTROL	10/95 REF. C.L.O.	15/95 REF. C.L.O.	20/95 REF. C.L.O.	25/95 REF. C.L.O.	15/100 COLL. C.L.O.	15/125 COLL. C.L.O.	15/150 COLL. C.L.O.	AVERAGE DIFFERENCE
1	.40	−.27	.28	.75	.21	.48	.36	.23	.37
2	.20	.10	−.50	.30	.20	−.40	−.10	.10	.24
3	−.56	.12	.48	.04	.55	1.05	.15	−.43	.42
5	.79	1.69	1.97	−.39	.00	.93	−.96	.90	.95
6	−1.02	1.43	.17	.34	−.90	−1.03	−1.48	−2.06	1.05
7	.10	−.80	−.50	−.50	1.30	.60	−.90	.40	.64
8	.13	.42	.49	−.23	.64	.31	1.04	.27	.44
9	−.23	−.18	.33	.80	1.08	−.28	−.58	.06	.44
10	−.23	.69	−.06	−.22	.08	.21	.11	−.02	.20
11	1.30	.00	1.50	.70	.60	.00	.10	.80	.63
12	−.25	.42	.52	.49	−.37		−.11	.18	.33
13	.40	−.80	.60	−.60	.10	1.10	1.20	1.10	.84

sponses to administration of vitamin D which were also among the lowest. It appears that variations in the calcium-phosphorus ratios of the test rations are definitely not the limiting factor in the accuracy of the method.

Suggestions have been made to standardize the A.O.A.C. ration on a definite calcium and phosphorus basis. This is a subject that should receive consideration in the future, but the Associate Referee does not consider it to be of extremely urgent importance at this time.

Average chick weights show a wide variation. For example, in the groups receiving 25 A.O.A.C. chick units of vitamin D per 100 grams of ration, the weights varied from 120 to 195 grams. In the negative control groups average weights varied from 104 to 150 grams. In spite of these wide variations in average weights obtained in the different laboratories they apparently had no effect on the final interpreted vitamin D potency of the collaborative sample.

Many of those now working with the method consider that a more normal growth is desirable. They believe that the test ration should be supplemented so as to produce better growth. Suggestions are that more yeast be added or possibly alfalfa meal. Opinion is quite general that the flavin content is sometimes too low for satisfactory growth. Further study of the relationship of growth to assay accuracy should be made.

COMPARISON OF COMPOSITE ASHING WITH INDIVIDUAL BONE ASHING

Table 3 shows the differences between composite ash of chick tibiae and the average of the ashes of the opposite individuals. The numbers given for each group, obtained from Table 1, represent the composite ash minus the average of the individual tibia ash. Thus, if the figure is preceded by the minus sign it indicates that the composite ash per cent was lower than the results obtained by individual bone ashings. It will be noted that some collaborators obtained far better agreement than others. It was suggested by one of the collaborators that possibly there was not complete ashing in the composite samples in 1 hour at 850° C. This suggestion is partly substantiated by the fact that in only 30 cases out of the possible 95 was the composite ash smaller than the average of the individuals. The figures given for Collaborator 9 are the result of two 1-hour ashings of the composites. He found a substantial lowering of the composite ash by ashing the additional hour. The Associate Referee ashed his composites for an additional 2-hour period, but was able to obtain only negligible differences. His muffle temperature was probably higher.

The average differences for the two ashing methods varied from .20 to 1.05 per cent. The excellent agreement obtained by some of the collaborators leads the Associate Referee to believe that some factor or factors other than the ashing technic is producing the variations. He suggests that one of these factors is the lack of uniform technic in cleaning

the opposite tibiae. The personal error is increased when more than one person is cleaning bones in the same series. Further study is necessary to clarify the reason for these discrepancies.

COMMENTS OF COLLABORATORS

The collaborators were asked for suggestions and a statement of general experiences with the method.

In general, the method appears to be satisfactory, but it would be far more acceptable if some of the inconsistencies in calcification responses could be eliminated. The calcifications obtained, plotted against vitamin D intake, do not always reveal the smooth curve that should theoretically be produced, which makes it impossible to interpret potency. One collaborator states that he is able to obtain a smoother curve with composite ashing. Another collaborator plots his response curve on semi-logarithmic graph paper in order to flatten the curve. Suggestions are made that a standard source for experimental chicks be established and that the flavin content of the ration be increased so as to obtain a greater and more uniform growth.

An essential need seems to be expressed generally for a standard method of interpretation of results. Several suggestions were made that interpretations be limited to only the steepest part of the response curve. One collaborator stated that he had noticed significant errors through unequal illumination of the feeding troughs. These comments do not exhaust the many constructive suggestions of the collaborators.

The Associate Referee is of the opinion that through conferences and experimental work during the next year enough evidence can be obtained so that a recommendation for a general revision of the method can be made and presented at the next meeting. He does not believe that the limit of accuracy with the general procedure has been obtained. No change in the tentative method or its status is recommended at this time.

The Associate Referee is grateful to the collaborators whose work is included in this report. Such collaborative work is both tedious and costly.

It is recommended that experimental and collaborative work be continued.

REPORT ON HYDROCYANIC ACID IN GLUCOSIDE BEARING MATERIALS

By ROBERT A. GREENE (Arizona State Laboratory, Tucson, Ariz.), *Associate Referee*

In accordance with the recommendations made by the Association last year, *This Journal,* **20,** 48 (1937), the investigations this year were planned to include a further study of the qualitative tests, the use of an

indicator solution, and a collaborative study of the photoelectric tubidimetric method.

Considerable difficulty was experienced in securing collaborators, and although samples were sent to all who signified a desire to collaborate, the demands of their regular work have not permitted all of them to make the examinations requested.

Collaborators were requested to make the qualitative and quantitative tests as directed in *Methods of Analysis, A.O.A.C.*, 1935, 347. In addition, they were requested to use, in the alkaline titration method, an indicator solution instead of adding the potassium iodide and ammonium hydroxide solutions separately. They were also requested, where equipment was available, to use the photoelectric tubidimetric method of Bartholomew and Raby.[1] The names of collaborators who were able to complete these examinations follow:

1. L. D. Haigh, University of Missouri, Columbia, Mo.
2. E. T. Bartholomew, Citrus Experiment Station, Riverside, Calif.
3. E. L. Breazeale, Arizona State Laboratory, Tucson, Ariz.
4. Gordon Nielson, Arizona State Laboratory, Tucson, Ariz.
5. R. A. Greene.

The results are given in Table 1.

TABLE 1.—*Results of collaborative tests* (mg. HCN per 100 grams sample)

COLLABORATOR	ALKALINE TITRATION METHOD* A	B	ACID TITRATION METHOD	QUALITATIVE TESTS
1	21.32	not made	22.41	not made
2	26.60	26.20	23.00	not made
3	28.35	28.35	21.60	positive
4	28.35	28.35	24.03	positive
5	28.35	28.35	21.99	positive
Averages	26.59	27.81	22 60	

* Method A is the use of the KI and NH_4OH solutions as recommended Method B employs a mixed indicator solution (1 ml. of a solution of 5 grams of KI, 5 ml. of NH_4OH, and 95 ml. of H_2O).

Although photoelectric equipment was not generally available, Bartholomew made these determinations by the method which he described in *This Journal*, **19**, 472, 589 (1936). The referee sample of flaxseed meal and a special sample of flaxseed (Punjab flax) from the Imperial Valley of California were employed. The results are given in Table 2.

The results given in Tables 1 and 2 show very good agreement. The results secured by Collaborators 1 and 2 were lower than those obtained in this laboratory when the alkaline titration method was used. Last

[1] *Ind. Eng. Chem., Anal. Ed.*, **7**, 68 (1935).

TABLE 2.—*Comparison of titration and photoelectric turbidimetric methods*
(Mg. HCN per 100 grams sample)

SAMPLE	ACID TITRATION	ALKALINE TITRATION* A	ALKALINE TITRATION* B	PHOTOELECTRIC TURBIDIMETRIC METHOD
Referee (Table 1)	23.00	26.60	26.20	24.05
Punjab flaxseed	38.15	41.22	38.92	38.95

* Method A is the use of the KI and NH_4OH solutions as recommended. Method B employs a mixed indicator solution (1 ml. of a solution of 5 grams of KI, 5 ml. of NH_4OH, and 95 ml. of H_2O).

year the same variation was noted, and the differences were chiefly attributed to loss of hydrocyanic acid during shipment or in storage prior to analysis. With one exception (Collaborator 1), the acid titration method gave lower results than did the alkaline titration method. Since the sensitivity of the methods (based upon the respective solubility products of silver iodide and silver sulfocyanate) are of the same order of magnitude, it would be expected that they should yield results showing agreement as obtained by Collaborator 1.

The results obtained in this laboratory are almost unbelievable. On the other hand, these collaborators had the advantage of using the same equipment and reagents. Furthermore, all determinations were made within a few days. Collaborators 3 and 5 have had much experience with these methods.

In general, there was no significant difference observed when the indicator was added as a single solution and when added separately. In view of the observations of Bartholomew, it seems preferable to add the indicator as a single solution. Bartholomew considers that when the method is carried out according to the official method excessive amounts of ammonium hydroxide are added, which influences results. Haigh has also pointed out that the amount of sodium hydroxide used to absorb the hydrocyanic acid may profoundly affect the results. It seems that it would be well next year to study these factors.

Table 2 gives the comparison of the several methods, including the use of the photoelectric turbidimeter. The agreement in the case of the Punjab flax is excellent; with the referee sample, the turbidimetric method gave lower results than did the alkaline titration method. In commenting on this, Bartholomew pointed out that the distillate from the Punjab flax was water clear, while that of the referee sample was turbid. In the latter case, Bartholomew considers that the results of the photoelectric method are correct.

The Associate Referee believes from this, as well as past collaborations with Bartholomew, that the photoelectric turbidimetric method is the most accurate. It has the further advantage of eliminating personal error in judging the end point.

TABLE 3.—*Effect of time of autolysis upon HCN liberated*
(Mg. HCN per 100 grams sample)

TIME	METHOD A*	Mg.HCN*	METHOD B
minutes			
0	16.87		16.87
30	23.97		23.97
hours			
1	27.00		26.32
2	28.35		27.67
3	28.35		27.67
5	27.67		27.67
6	27.95		28.35
7	28.35		28.35
12	28.35		28.35
24	27.00		27.00

* In Method A, the KI and NH_4OH solutions were added separately, in Method B, they were added as an indicator.

Table 3 shows that at the end of 2 hours the amount of hydrocyanic acid present is practically at a maximum, and it seems that beyond that time there is no significant difference. As a routine procedure, it seems that the time should be arbitrarily set at 2–4 hours. There is some variation in results obtained by the two methods, but actually the extreme difference between the titrations by the two methods was 0.05 ml. of silver nitrate.

The acid titration method has given satisfactory results. The principal objection is well stated by Haigh: " . . . it requires the use of two standard solutions instead of one. The filtration which is necessary of course increases the possibility of variation in results."

The qualitative test of Fox[1] has been used in this laboratory, and it has given satisfactory results. Its sensitivity and specificity are much greater than the Guignard test, but the simplicity of the latter is a factor favoring its use. The technic of the Fox test is given in the Report on Hydrocyanic Acid in Plants and will not be repeated here. It is highly recommended as a routine test.

The Associate Referee gratefully acknowledges the assistance and cooperation of the collaborators.

RECOMMENDATIONS[2]

It is recommended—

(1) That the modified alkaline titration method be adopted as official.

(2) That the acid titration method be adopted as official.

(3) That the photoelectric turbidimetric method of Bartholomew and Raby be adopted as official.

[1] *Science*, **79**, 37 (1936).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, **21**, 60 (1938).

(4) That the qualitative (picrate) test be adopted as official.

(5) That the Fox test be adopted should another qualitative test be desired.

(6) That the effects of various concentrations of sodium hydroxide (alkaline titration method) be studied.

(7) That the use of a single indicator solution be given further study.

REPORT ON FAT IN FISH MEAL

By R. W. Harrison (U. S. Bureau of Fisheries, Seattle, Wash.), *Associate Referee*

Due to the pressure of other duties and demands on the facilities for extraction in the course of normal investigational work, it has been impossible to adhere closely to the program for study outlined in last year's report. In fact, the data obtained to date can be considered only as being of a preliminary nature for the purpose of indicating the gross extraction power of several types of solvents. This information, however, brings out several interesting points and therefore is being reported briefly at this time.

During the year over 200 individual determinations were made, involving 4 samples of meal, 12 different solvents, and extraction periods ranging from 4 to 32 hours. The data obtained are given in Tables 1 and 2. The purpose of these determinations was to find the rate at which the various solvents approached a maximum extract value, the gross extract value of the solvents on the meals as received, and the gross extract value of the solvents after the meals had been subjected to storage conditions that would lead to oxidation of the fat. In this connection the meals as received had passed through a certain degree of oxidation following preparation, and the initial data, therefore, cannot be considered as representative of the meal or the action of the solvents on the meal at time of preparation. To date only one sample has been examined after further oxidation in the laboratory. In this case the conditions for oxidation were not severe and the storage period was only for a short time.

In making the determinations the meal samples were dried in a vacuum oven for 2 hours at 80° C. This uniform drying period was used in preference to attempted drying to constant weight, because experience has shown in the case of fish meals that once the meal is completely desiccated subsequent drying leads to increase in weight, and the 2-hour period was found adequate for obtaining a minimum value.

Because of the wide variation in the boiling temperatures of the solvents the heat was regulated so as to give approximately a uniform interval of syphoning of the extraction chamber of the Soxhlet apparatus

TABLE 1.—*Per cent extract for different extraction periods*

SOLVENT	B.P. °C.	MEAL NO. 1 8 HRS.	16 HRS.	24 HRS.	MEAL NO. 2 4 HRS.	8 HRS.	16 HRS.	24 HRS.	32 HRS.
Petroleum	35–60	13.7	13.6	13.6	7.5	7.2	7.3	7.3	6.7
Ether			13.6		7.1	7.2		7.0	
					7.2			7.2	
Hexane	62-67						7.1	7.2	7.1
(pract.)							7.1		
Heptane	91–96					7.2	7.2	7.4	7.3
(pract.)						7.4	7.1		
						7.2	7.1		
Ethyl	34.5	14 5	14.7	14.6	7.5	7 5	7.9	8.0	7.6
Ether			14.3		7.6	7 5		7.5	
					7.6	7.7		7.6	
Carbon	46.3	14.3	14.4	14.4	7.7	7.8	7.9	8.2	7.5
Bisulfide			14.1		7.7	7.8		7.7	
					7.7			7.7	
Cyclohexane	78-81					7 5	7.8	7.7	7.5
						7.6	7.4		
						7.7	7.4		
Benzene	79.6	15.2	15.1	14.8	7.8	8.1	8.4	8.4	8 0
			15.3		7.9	8.2			
					7.9	8.2			
Methylene	40–41					8.1	8.3	8.6	8.4
Chloride							8.5	8.3	
								8.5	
Trichloro-	83–87					8.4	8.6	8.9	8.8
ethylene						8.7	8.5		
						8.8	8.4		
Chloroform	61.2	16.5	16.3	16.4	9.1	9.1	9.3	9.4	9.0
			16.7		9.2	9.1		9.2	
					9.0			9.1	
Acetone	56.1	16.1	16 4	16.9	8.3	8.7	9.4	9 8	9.3
			16.4		8.4	8.8		9.0	
					8.5	8.8		9 2	
1–4 Dioxane	91–101					11.4	10.9	14.8	12.2
						11.6	11.3	11.9	
						11.5		12.4	

TABLE 2.—*Solvent extract values—24 hour extraction*

SOLVENT	B.P. °C.	PER CENT SOLVENT EXTRACT MEAL 1	MEAL 1 OXIDIZED SLIGHTLY	MEAL 2	MEAL 3	MEAL 4
Petroleum Ether	35–60	13.6	11.0	7.3	8.3	8.8
				7.0	7.5	9.1
				7.2	7.5	8.5
Hexane (pract.)	62–67		11.8	7.2	7.5	10.2
					7.4	9.7
					7.5	9.4
Heptane (pract.)	91–96		12.1	7.4	7.7	9.7
					7.8	10.5
					7.7	10.2
Ethyl Ether	34.5	14.6	13.1	8.0	7.8	9.7
			12.8	7.5	8.0	10.4
				7.6	7.7	
Carbon Bisulfide	46.3	14.4	12.9	8.2	8.4	10.5
				7.7	8.0	10.4
				7.7	7.9	
Cyclo-hexane	78–81		13.0	7.7	8.0	10.4
					8.2	10.5
					8.0	
Benzene	79.6	14.8	14.4	8.4	8.4	11.0
			14.1		8.6	11.0
					8.3	
Methylene Chloride	40–41		14.2	8.6	9.0	11.4
				8.3	8.8	11.3
				8.5	8.5	
Trichloro-ethylene	83–87		16.3	8.9	9.1	11.5
					9.2	11.8
					9.2	11.6
Chloroform	61.2	16.4	15.1	9.4	9.1	11.8
				9.2	9.0	11.7
				9.1	9.3	
Acetone	56.1	16.9	16.2	9.8	8.9	11.9
				9.0	9.2	11.8
				9.2	8.8	
1–4 Dioxane	91–101		19.0	14.8	11.4	15.8
				11.9	10.7	17.6
				12.4	12.0	15.3

used. The interval between syphonings varied between 16 and 19 minutes, with 100 cc. of solvent being used in each case. At the end of the extraction period the greater portion of the solvent was distilled from the extraction flask and the flasks were heated to a minimum weight in the vacuum oven at 80° C. The determinations were made six at a time, and six solvents were used. None of the data reported represents duplicates or triplicates run simultaneously.

The data obtained show that of the twelve solvents studied, all except acetone gave very little if any increase in extraction value beyond 4 hours. In the case of acetone 16–24 hours were required to reach a maximum value.

From the standpoint of gross extraction the supposedly aliphatic petroleum hydrocarbons gave the lowest values, and these in general increased with boiling temperature. Ethyl ether, carbon bisulfide, and cyclohexane gave quite similar values, slightly above petroleum ether, hexane, and heptane.

It is of interest that cyclohexane, which is a closed chain compound, gives a value intermediate between the open chain solvents and benzene, which is definitely a ring compound. Likewise, the chlorinated aliphatic hydrocarbon solvents are found together on basis of extraction value and give results higher than the unsubstituted chain or ring hydrocarbons. In the initial determinations on the meals, chloroform gave values quite similar to those given by acetone but was less effective on the oxidized meal. The cyclic ether 1,4 dioxane gave the highest values of the solvents tested.

Only casual comment can be given in regard to the extraction properties of the several solvents on oxidized meal since but one sample was examined, and all solvents were not used on both the initial and the subsequently oxidized materials. These data do indicate, however, the necessity of the present investigation, since the initial petroleum ether extract value decreased from 13.6 to 11.0 per cent due to the slight oxidation, while the ethyl ether extract value decreased from 14.6 to about 13 per cent. These data likewise demonstrate the discrepancy in results where fat content is based in some cases on ethyl ether extraction and in other cases on petroleum ether extraction.

Meal samples 3 and 4 have been subjected to extended oxidation and will be examined shortly. When these data have been obtained, the extracts of the solvents showing most promise will be examined chemically and investigation of these solvents will be continued.

REPORT ON BIOLOGICAL METHODS FOR VITAMIN B COMPLEXES

By C. A. ELVEHJEM (Department of Agricultural Chemistry, University of Wisconsin, Madison, Wis.), *Associate Referee*

Previous reports, *This Journal*, **18**, 354 (1935); **19**, 595 (1936), have included studies on a proposed method for the determination of vitamin B_1 and the use of this method for the estimation of vitamin B_1 in several samples of yeast. The method has been used continuously in this laboratory with good results. One slight change has been made to insure an adequate supply of vitamin A. In addition to the basal ration (242A), each chick was given two drops of halibut liver oil twice weekly.

TABLE 1.—*Results with 4 chicks receiving different levels of brewers' yeast 132.*

GROUP NUMBER	SUPPLEMENT—BREWERS' YEAST 132	CHICKS SURVIVING 5 WEEKS	WEIGHT AT 5 WEEKS	TIME OF POLYNEURITIS
	per cent		*grams*	*days*
471	0.40	4	81	none
472	0.45	4	82	none
473	0.50	4	142	none
488	0.30	none	—	9–21
489	0.35	3	122	10
490	0.40	2	108	none
510	0.35	1	73	14–21
511	0.40	3	107	none
512	0.45	4	114	none
538	0.30	2	124	18–25
539	0.35	3	107	27
540	0.40	4	114	none
541	0.45	4	115	none
552	0.35	2	120	18–22
553	0.40	4	108	none
554	0.45	3	153	none

During the past year attempts were made to establish the vitamin B_1 requirements of chicks when fed ration 242A. Previously the vitamin content of all samples was calculated by comparing the response of the chicks when receiving the unknown sample with that obtained when a standard sample of yeast was fed. These studies have been simplified due to the availability of larger samples of the International Standard acid clay adsorbate and crystalline vitamin B_1.

There is some question about the method of expressing the vitamin requirement since the activity of this factor is related to both body weight and carbohydrate metabolism. An expression per unit weight of feed is most valuable from a practical point of view. At some stages of growth

the vitamin B_1 intake on this basis will be higher than the requirement, but the method will measure the requirement at the most critical period. This procedure may be adapted readily to a determination of the antineuritic potency of vitamin B_1-containing substances.

In order to determine possible variations in different batches of chicks the response of chicks to different levels of a standard sample of brewers' yeast was studied first. Levels ranging from 0.30 to 0.50 per cent were fed. Brewers' yeast* was used instead of bakers' yeast because much smaller quantities were required. The results presented in Table 1 demonstrate that the requirement is remarkably constant. In five separate assays it was found that all the chicks in Groups 471, 490, 511, 540, and 553 were protected from polyneuritis when the basal ration was supplemented with 0.4 per cent brewers' yeast 132. When the level of yeast was reduced to 0.35 per cent, one or more chicks in Groups 489, 510, 539, 552 died after exhibiting the typical symptoms of polyneuritis.

TABLE 2.—*Results with 4 chicks receiving different levels of the International Standard*

GROUP NUMBER	SUPPLEMENT—INTERNATIONAL STANDARD	CHICKS SURVIVING 5 WEEKS	WEIGHT AT 5 WEEKS	TIME OF POLYNEURITIS
	per cent		*grams*	*days*
569	0.20	4	101	none
570	0.225	4	113	none
591	0.15	none	—	10–14
592	0.175	none	—	14–18
593	0 20	4	104	none
604	0.175	1	90	18–19
605	0.20	3	112	none
606	0.225	4	137	none

It has been found that chicks are able to elute the vitamin from the International Standard acid clay adsorbate. The results obtained by feeding different levels of this standard are shown in Table 2. When the chicks were fed Ration 242 supplemented with 0.175 per cent International Standard, all chicks in Group 592 died, two showing polyneuritis, and two of the four chicks in Group 604 died after exhibiting the typical symptoms of polyneuritis. All the chicks in Groups 569, 593, and 605 were protected from polyneuritis when fed Ration 242A supplemented with 0.20 per cent International Standard. An improved rate of growth resulted when the chicks were fed Ration 242A supplemented with 0.225 per cent International Standard (Groups 570 and 606). When the criterion for the determination of the minimum protective level is taken to be protection from polyneuritis for all the chicks in the group it is evident that the vitamin B_1 requirement of the chick is constant.

* The writer is indebted to Dr. Harold Levine of the Premier-Pabst Corporation, Milwaukee, for a generous supply of yeast.

The requirement was also established in terms of the crystalline vitamin. Two accurately weighed samples of crystalline vitamin B_1 hydrochloride (synthetic) were dissolved in 20 per cent alcohol to give a final concentration of 100 micrograms per cc. For feeding purposes, suitable aliquots were dried in vacuo on about 50 gram portions of the autoclaved component of the ration. This was ground by hand before incorporation into the ration, care being taken to eliminate losses.

TABLE 3.—*Results with chicks receiving different levels of crystalline vitamin B_1*

GROUP NUMBER	SUPPLEMENT—SYNTHETIC VITAMIN B_1HCl (1)	NUMBER OF CHICKS	CHICKS SURVIVING 5 WEEKS	WEIGHT 5 WEEKS	TIME OF POLYNEURITIS
	per cent			*grams*	*days*
581	0.00006	5	none	—	13–18
582	0.00007	5	1	100	13–27
583	0.00008	4	4	144	none
584	0.00006 (2)	4	4	115	none
585	0.00007	5	5	145	none
586	0.00008	4	4	174	none

Typical results with the two samples of vitamin B_1 hydrochloride are shown in Table 3. Protection of the chicks from polyneuritis resulted when they were fed Ration 242A supplemented with 80 micrograms of vitamin B_1 hydrochloride (Sample 1) per 100 grams of ration (Group 583). Lower levels of this product did not protect all the chicks from polyneuritis (Groups 581 and 582). When the chicks were fed the basal ration supplemented with 60 micrograms of vitamin B_1 hydrochloride (Sample 2) per 100 grams of ration, complete protection from polyneuritis resulted (Group 584). The wide variation in the growth rates of the chicks in this group was due to the fact that this was a borderline protective level. When the vitamin B_1 in the ration was increased slightly growth was improved (Groups 585 and 586).

DISCUSSION

The results in this report, as well as other data obtained in this laboratory, indicate that the vitamin B_1 requirement of chicks based on unit weight of feed is constant. On the basis of the results obtained with the International Standard it is seen that the requirement is between 20 and 25 International Units per 100 grams of Ration 242A. Twenty International Units protected against polyneuritis with several different groups of chicks on the specific basal ration used. Somewhat better growth resulted with 25 International Units per 100 grams of Ration 242A. It is perhaps safer to assume that the normal requirement is about 25 Inter-

national Units since Ration 242A is known to contain slight amounts of vitamin B_1. A slightly higher level may be necessary on a basal ration completely devoid of this vitamin.

A significant difference in the antineuritic potency was observed between the two samples of crystalline vitamin B_1 hydrochloride that were tested. Sample 1 was more active since 60 micrograms per 100 grams of Ration 242A protected the chicks from polyneuritis. Eighty micrograms of Sample 2 per 100 grams of Ration 242A were required to protect the chicks from polyneuritis. The reason for the variation in antineuritic potency is not clear at the present time. Nevertheless, there seems to be justification in assuming that Sample 1 yields a truer evaluation of the antineuritic potency of the crystalline vitamin.

The above data permit an evaluation of the antineuritic potency of brewers' yeast 132. Since 0.40 per cent of brewers' yeast 132 was required to protect the chicks from polyneuritis, it follows that this yeast sample contained 50 International Units per gram. Similarly, a comparison between the International Standard and vitamin B_1 hydrochloride (Sample 1) indicates that vitamin B_1 hydrochloride contained 330 International Units per mg.

The establishment of the values for the minimum vitamin B_1 requirements of chicks will greatly simplify the calculations of the vitamin B_1 content of food materials where chicks have been used for the assays. The results obtained with the chicks indicate that this method possesses certain advantages over the rat assay method. Less difficulty is experienced in the preparation of the basal ration for chicks than in the purification of materials for synthetic rat rations. The data obtained with several different batches of chicks show that no difficulty is encountered with respect to storage of vitamin B_1 in the day-old chick. In addition it appears that the chick is more sensitive than the rat to small additions of the vitamin to the basal ration.

RECOMMENDATIONS[1]

It is recommended—

(1) That the proposed biological method for the determination of vitamin B_1 in feeding stuffs be given further consideration with a view to its adoption as a tentative method.

(2) That the method be studied further collaboratively.

No report on biological testing for vitamin B complexes was given by the associate referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 21, 60 (1938)

REPORT ON CAROTENE

By V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year three methods, the Fraps revision of the Guilbert method, the Hughes-Peterson revision of the Guilbert method, and the U. S. Dairy Industry method, were sent out for collaborative study for the determination of carotene in alfalfa leaf meal and a commercial mixed feed containing considerable alfalfa leaf meal. The carotene solutions for all methods were compared against 0.1 per cent potassium dichromate solution and the results calculated from the conversion table given in the Fraps procedure, *This Journal*, **20**, 459 (1938). Since some unfavorable comment was made last year on the 0.1 per cent potassium dichromate, the work this year included the application of two other commonly used standards, the 0.036 per cent potassium dichromate solution proposed by Russell,[1] and the dye standard proposed by Guilbert, referred to in this study last year.

The carotene determination consists of two distinct problems, one the isolation and separation of the carotene, and the other the measurement of the extracted carotene in solution. In order to determine the variation in the actual measurement of the isolated carotene solution and also to make the best selection of the above standards, two samples of carotene in oil were submitted to the collaborators this year. They were instructed to prepare the carotene solution from a definite amount of oil and to measure the concentration of the carotene according to the three standards referred to above. Sample 1 contained only carotene and needed only to be weighed, made to volume, and matched against the standard. Sample 2 was a commercial oil with added carotene plus some xanthophyll, therefore it was necessary to follow the procedure for the separation of xanthophyll before matching the carotene. In spite of the precaution taken to use a freshly purchased lot of coconut oil, Sample 1 became bleached to such an extent that the collaborative results on this sample were of no value, and therefore the desired information on the agreement to be expected by simply matching against the various standards on a carotene solution was not obtained. The results on Sample 2 do not show the agreement to be expected on matching the oils with the various standards, but they do show the combined effect of the xanthophyll separation and subsequent matching against the standards. The results on the oil are given in Table 1.

The variation in results (Table 1) is greatest for the dye and least for the 0.036 per cent potassium dichromate, as is also the average measured amount of pigment. The 0.1 per cent potassium dichromate result was

[1] *Plant Physiol.*, **10**, 325 (1935).

TABLE 1.—*Results on amount of carotene in oil sample*
(Expressed in p.p.m.)

COLLABORATOR NO.	0.1% $K_2CR_2O_7$	0.036% $K_2CR_2O_7$	DYE	SPECTROPHOTOMETER
1	53.5	48.1	60.9	50.0
2	48.2	45.0	49.0	—
3	50.3	44.6	45.3	—
4	45.9	51.1	56.9	—
5	57.5	51.8	59.1	51.5
Av.	51.0	48.2	54.2	50 8
Max.	57.5	51.8	60.9	51 5
Min.	45.9	44.6	45 3	50.0
Range	11.6	7.2	15.6	1.5

intermediate and most closely agreed with the spectrophotometric result.

The results on the oil samples were supplemented by those on a sample of alfalfa leaf meal which the collaborators were requested to analyze for carotene by the Fraps revision of the Guilbert method, the Hughes-Peterson revision of the Guilbert method, and the U. S. Dairy Industry method, slightly revised since last year. This modified method follows:

The method is usable with materials that readily grind completely in a Wiley mill. It is particularly useful with the hays. The bales are bored repeatedly with a 2-inch drill. The drillings are ground through the medium (1 mm. holes) and then through the finest (0.5 mm. holes) sieve in a Wiley mill. Aliquots are taken for analysis. Absolute ethanol (15 ml. for a 6 gram sample) is added; and, after standing 1 hour or more (overnight), the samples are extracted repeatedly (6 times or more) by shaking (15 minutes) with low-boiling (30°–60° C.) petroleum ether (20 ml. portions for a 6 gram sample). A sintered glass funnel is used in removing the extract. The combined extract is concentrated in vacuo to 75–100 ml.; 20 ml. of water is added; and after the mixture has been shaken 2–5 minutes the alcohol-water layer is discarded. The chlorophyll and about 95% of the xanthophyll are removed from the carotene by two treatments of the ligroin fraction with a saturated KOH methanol (10 ml. each time) solution. The mixture is shaken 15 minutes with each treatment; the alcoholic layer is then diluted with 1 ml. of water, the shaking is continued for 2–5 minutes more, and the diluted alcoholic potash layer is discarded. The petroleum ether fraction is then washed free of xanthophyll by shaking it repeatedly for 2 minutes with 25 ml. portions of 92 per cent methanol (by volume). The methanol used in the final washing should be colorless. The carotene fraction is then concentrated in vacuo and brought to volume with a mixture (1:1) of ethanol and higher boiling (about 90° C.) petroleum ether. The ethanol retards the oxidation of the carotene.

The collaborators were also requested to match the solutions from all the methods against the same three standards used on the oils, and by the spectrophotometer if available. The results on the alfalfa samples are given in Table 2.

The average results obtained by the three methods when the same standard was used are highest when compared against the dye standard,

TABLE 2.—*Results on amount of carotene in alfalfa leaf meal*
(Expressed in p.p.m.)

COLLABORATOR NO.	FRAPS REVISION OF GUILBERT METHOD				HUGHES-PETERSON REVISION OF GUILBERT METHOD				REVISED DAIRY INDUSTRY METHOD			
	DYE[1]	0.1% $K_2CR_2O_7$	0.036% $K_2CR_2O_7$	SPECTRO-PHOTOMETER	DYE	0.1% $K_2CR_2O_7$	0.036% $K_2CR_2O_7$	SPECTRO-PHOTOMETER	DYE	0.1% $K_2CR_2O_7$	0.036% $K_2CR_2O_7$	SPECTRO-PHOTOMETER
1	170	180	170	—	170	180	170	—	157	170	157	—
2	172	142	144	145	193	155	158	159	234	188	190	183
3	175	147	147	—	179	148	152	—	203	175	161	—
4	169	166	165	169	166	174	166	171	170	170	163	168
5	201	199	175	176(179)[2]	217	200	186(183)[2]	176	231	203	189	182(185)[2]
6	229	174	174		216	161	178		188	182	166	
7	197	190	167		195	191	173		182	175	153	
Av.	188	171	163	163	191	172	169	169	195	181	168	178
Max.	229	199	175	176	217	200	185	176	234	203	190	183
Min.	169	142	144	145	166	148	152	159	157	170	153	168
Range	60	57	31	31	51	52	33	17	77	33	37	15

[1] Dye standard submitted for use in this work agreed with Guilbert's dye standard according to his measurements.
[2] Photoelectric colorimeter.
Note: All results are the average of duplicate determinations, with the exception of those of Collaborator 2, which are the average of each of three analysts' results read in duplicate.

next the 0.1 per cent potassium dichromate, and lowest against the 0.036 per cent potassium dichromate. The average results for the three methods when the collaborators used the same standard are in very good agreement, indicating that the methods of extraction give about the same results. The variations shown are due largely to the standards, such as the inaccuracy of the conversion factor, improper application, or difficulty in matching. They are greatest for the dye standard, next for the 0.1 per cent potassium dichromate, and smallest for the 0.036 per cent potassium dichromate. The least spread in the results is observed with the use of the spectrophotometer. The revised Dairy Industry method gave, on the average, slightly higher results. These conclusions on the alfalfa sample are in agreement with those on the oil with reference to the standards. The results for the dye standard are apparently not only too high, but they also show that the analysts obtained greater variation. The results by the 0.036 per cent and the 0.1 per cent potassium dichromate standard are about equally close to those obtained by the spectrophotometer, but the 0.036 per cent potassium dichromate shows the least variation.

The spectrophotometer is the most reliable means of measuring carotene in solution, and consequently these results (Table 3) may be considered closest to the true value. However, it should be emphasized that the spectrophotometric results will differ according to the wave length at which the absorption coefficient is determined. It is for this reason that Hughes and Peterson recommend the determination of the concentration of the solution at the three wave lengths, namely, 450, 470, and 480 mμ, and the reporting of the average. This average will be fairly constant for the same product and will probably be more nearly the true value. This variation in concentration at different wave length settings is probably due to the fact that the extracted carotene solution obtained by the present methods does not follow the absorption curve for pure beta carotene from which the conversion factors are obtained. Collaborator 2 shows the variation obtained at different wave length settings by the three different methods. The results of only one determination are reproduced here.

TABLE 3.—*Spectrophotometric results on amount of carotene in alfalfa obtained at different wave lengths*

(Expressed in p.p.m.)

METHOD	455 mμ	470 mμ	480 mμ	AVERAGE
Revised Dairy Industry method	188.8	180.8	174.0	181 2
Fraps revision of Guilbert method	153.0	146.0	139.0	146.0
Hughes-Peterson revision of Guilbert method	164.2	158.4	147.5	156.7

The variation here (Table 3) is about 14–17 p.p.m. The Associate Referee has obtained a variation as great as 20–25 p.p.m. on alfalfa when the concentrations were measured at wave lengths 450, 470, and 480 mμ.

In Table 4 Peterson shows the variation in the optical densities at the three commonly used wave lengths for a number of products.

TABLE 4.—*Ratios of optical densities of carotene solutions at wave lengths 4550, 4700, and 4800 Å.U., O.D. at 4700 Å. = 1.00**

NO. OF SAMPLES	PRODUCT	4550 Å	4700 Å	4800 Å
5	Beta Carotene	1.18	1.00	1.05
6	Butter	1.14	1.00	1.04
42	Egg Yolk	1.17	1.00	.92
46	Dehydrated Alfalfa	1.19	1.00	1.05
16	Prairie Hay	1.38	1.00	1.03
20	Spring Grasses	1.18	1.00	1.03
21	Summer Grasses	1.19	1.00	1.03
11	Fall Grasses	1.29	1.00	1.06
3	Silage	1.23	1.00	1.00
6	Yellow Corn	1.16	1.00	.87
27	Misc. Commercial Feeds	1.20	1.00	.96

* For ready comparison average optical densities at each of the three wave lengths have been calculated to show their relationship to the optical density at 4700 Å when it equals 1.00.

Each collaborator was asked to comment on the choice of the method of extraction and the standard. Five of the collaborators preferred the Hughes-Peterson modification of the Guilbert method, and they were equally divided on the 0.1 per cent and 0.036 per cent potassium dichromate standards. The choice of this method of extraction is apparently largely based on its speed and convenience. Since the principles of none of the three methods allow the determination of pure carotene the choice of the method of extraction cannot be made on this basis. All the information available indicates that the Hughes-Peterson procedure is preferable and should be subject to further collaborative study next year, with more detailed directions. The dye standard received unfavorable comment on the basis that variation occurs in different batches of dye and that the dye standard may not be permanent, and these disadvantages are not balanced by sufficiently increased facilitation of reading. In support of the dye standard Guilbert reports that the dyes appear to be uniform, that the dye solutions are stable over a period of two years, and that a linear relationship exists between concentration and depth compared with carotene. However, on the basis of the comments of the collaborators and the results reported, the dichromate standards seem more desirable. The 0.1 per cent dichromate seems somewhat better for the more concentrated solutions, while the 0.036 per cent dichromate is better for the more dilute solutions. Both of these standards are stable, easy to make, and should be further studied in connection with this work. The collaborative results and comments of the collaborators indicate that the spectro-

photometer is the most accurate and convenient instrument for carotene measurements, but these instruments are not generally available and therefore study on the use of the colorimetric method should be continued.

Favorable results were obtained by Collaborator 5 with the photoelectric photometer and by the Associate Referee with application of the neutral wedge photometer described in *This Journal*, **19**, 150, (1936), by Clifford and Wichmann in their work on lead determinations.

This report is possible through the very generous cooperation of the following collaborators, to whom many thanks are due:

H. R. Guilbert and R. W. Caldwell, University of California, Davis, Calif.

L. M. Kishlar, V. Wodicka, and W. Brew, Ralston Purina Co., St. Louis, Mo.

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W. J. Peterson, Kansas State College of Agriculture and Applied Science, Manhattan, Kan.

G. S. Fraps and A. R. Kemmerer, Agriculture and Mechanical College, College Station, Texas.

M. W. Taylor, Agricultural Experiment Station, New Brunswick, N. J.

RECOMMENDATIONS[1]

It is recommended—

(1) That the Hughes-Peterson procedure, with more detailed directions, be submitted to further collaborative study.

(2) That the 0.1 per cent and the 0.036 per cent potassium dichromate standards be rechecked against pure beta carotene and the best conditions for accurate application be established and used as the colorimetric standards for the Hughes-Peterson procedure.

(3) That study of the application to the carotene determination of the neutral wedge photometer and the photoelectric colorimeter be continued.

REPORT ON QUALITATIVE TESTS FOR PROTEIN

By D. Breese Jones (Bureau of Chemistry and Soils, Washington, D.C.), *Associate Referee*

Inasmuch as the tests for protein have been in general use for many years and are well established, it is not believed that much would be gained by further work on them.

It is, therefore, recommended[1] that these tests as previously reported be made official (final action).

The proceedings for Wednesday afternoon, the reports of the committees, etc., will be found in Volume **21**, No. 1.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **21**, 60 (1938).

CONTRIBUTED PAPERS

THE DEHYDROGENATION OF ALKALI LIGNIN FROM CORN COBS WITH SELENIUM*

By MAX PHILLIPS and M. J. GOSS

Dehydrogenation by means of selenium has been of greatest value in elucidating the structures of many naturally occurring complex organic substances, (1) such as polyterpenes, sterols, bile acids, sex hormones, alkaloids, and toad poisons. Wedekind (2) applied this method of dehydrogenation to so-called dioxane lignin from ebony, but his results were of an entirely negative character.

The present paper gives results of some experiments on the dehydrogenation of alkali lignin from corn cobs with selenium in an atmosphere of nitrogen. The oil obtained from several dehydrogenation experiments consisted of a mixture of phenolic substances, among which guaiacol and 1-n-propyl-3-methoxy-4-hydroxybenzene were identified, together with organic selenium-containing substances. No hydrocarbon that would throw additional light on the structure of lignin could be isolated.

EXPERIMENTAL

Apparatus.—The apparatus used was similar to the one described in a previous communication (3). The glass reaction tube (Pyrex) was filled as follows: A plug of glass wool was placed at the constricted end of the tube. This was followed with a 5 cm. layer of pumice impregnated with selenium (prepared by adding pumice of about the size of peas to a thin suspension of 20 grams of selenium in water, mixing thoroughly, and then drying the pumice at 105° C.). After the pumice-selenium layer, an intimate mixture of 50 grams of lignin, 100 grams of selenium, and a few pieces of pumice were added. Following this there was added another 5 cm. layer of pumice impregnated with selenium, and finally a plug of glass wool.

Procedure.—Fifty grams of alkali lignin from corn cobs (prepared as described in a previous communication (4) except that the hydrolysis with the acid was conducted for 2 hours) was intimately mixed with 100 grams of selenium and placed in the reaction tube prepared as above described. The air in the apparatus was first replaced with dry nitrogen, and then the temperature of the tube was gradually raised to 300° C. in the course of 3 hours. Finally, the temperature was increased to 340° C. and maintained at that level for 2 hours. A slow stream of dry nitrogen was passed through the apparatus throughout the distillation experiments. A distillate was obtained which consisted of an aqueous portion plus a brown oily portion. The black carbonized material remaining in the tube

* Contribution No. 289 from the Industrial Farm Products Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

after each distillation experiment was extracted with ether. After removal of the ether by evaporation, the oily residue was added to the main distillate. Five such experiments were carried out, representing a total of 250 grams of lignin. The distillates plus the ether extracts of the carbonized material from the five experiments were combined and dissolved in ether; the ether solution was dried over anhydrous sodium sulfate and filtered; and the ether was then removed by evaporation. The brown oil obtained amounted to 32.41 grams, 12.9 per cent of the weight of moisture-free lignin used in the five experiments.

Examination of the Oil.—The oil was subjected to steam distillation until no more came over in the distillate. The oil in the distillate was separated; the aqueous portion was extracted with ether; and the ether extract was added to the oily distillate. The weight of this oil amounted to 20.48 grams, 8.2 per cent of the weight of the lignin used in the distillation experiments. It was a light-brown oil (A).

The fraction not volatile with steam (B) amounted to 5.16 grams, 2.0 per cent of the weight of the lignin used. It was a dark-brown viscid product.

Examination of the Steam-Volatile Fraction (A).—The ether solution containing this oil fraction was successively and exhaustively extracted with a 5 per cent aqueous sodium bicarbonate solution and a 5 per cent aqueous sodium hydroxide solution. The ether solution remaining after the extraction operation contained the neutral fraction (C).

Examination of the Sodium Bicarbonate Extract.—The sodium bicarbonate extract was acidulated with sulfuric acid and distilled in a current of steam until the fresh distillate no longer reacted acid to litmus. The distillate was titrated with 0.1 *N* sodium hydroxide solution, and 12.0 cc. was required (0.072 gram of acid calculated as acetic acid). This quantity represented 0.03 per cent of the weight of the lignin used in the distillation experiments.

Examination of the Sodium Hydroxide Extract.—The sodium hydroxide extract was acidified with sulfuric acid and extracted with ether. The ether solution was dried over anhydrous sodium sulfate and filtered, and the ether was removed by evaporation. The oily residue obtained amounted to 8.66 grams, 3.5 per cent of the weight of the lignin used in the distillation experiments. This oil was fractionally distilled and separated into the following three fractions:

	°C.	grams
(1)	200–210	1.84
(2)	210–215	2.32
(3)	215–240	1.16

The 3,5-dinitrobenzoyl derivatives of the three phenolic fractions were prepared according to the method described in a previous communication (5).

The 3,5-dinitrobenzoyl derivative of fraction 200–210° C. was identified by the melting point, mixed melting point, and the optical properties* of the crystals as guaiacol.

The 3,5-dinitrobenzoyl esters of fractions 210–215° and 215–240° C. were identified as 1-n.-propyl-3-methoxy-4-hydroxybenzene (identified by melting points and by the optical properties of the crystals).

Steam Volatile Neutral Fraction.—The ether solution of the steam-volatile fraction remaining after successive extractions with sodium bicarbonate and sodium hydroxide solutions was dried over anhydrous sodium sulfate and filtered, and the ether was evaporated. An oil having a very offensive odor was obtained. The weight of this oil amounted to 7.90 grams, 3.2 per cent of the weight of the moisture-free lignin used. The oil was distilled under ordinary pressure, and most of the material distilled at 150–170° C. The weight of the distillate was 6.78 grams. The oil was then fractionally distilled under ordinary pressure and resolved into two fractions: (1) 150–155° C.—3.40 grams; (2) 155–160° C.—2.50 grams.

The percentages of carbon and hydrogen were determined, and the following results were obtained:

Fraction 150–155° C.—Found: C, 14.57, 14.56; H, 3.38, 3.32.

Fraction 155–160° C.—Found: C, 14.39, 14.26; H, 3.36, 3.33.

Both fractions contained selenium and appeared to be mixtures of selenides. The percentage of selenium in fraction 155–160° C. was determined. The material was first fused in the Parr bomb with sodium peroxide, the procedure generally employed in the determination of sulfur in organic substances being followed. The cold melt was dissolved in water, and the selenium was determined in this solution by the method of Robinson, Dudley, Williams, and Byers (6). The following results were obtained.

Found: Se, 80.77 and 80.65.

Fractions 150–155° and 155–160° C. when oxidized with potassium permanganate following the procedure described in a previous communication (7) afforded no anisic acid.

Examination of the Steam-Nonvolatile Fraction.—The ether solution of this fraction was successively extracted with a 5 per cent sodium bicarbonate solution and a 5 per cent sodium hydroxide solution. The ether solution remaining after these extractions contained the steam-nonvolatile neutral fraction.

The sodium bicarbonate extract was acidulated with hydrochloric acid

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* All identifications of compounds by optical methods were made by G. L. Keenan of the Microanalytical Laboratory of the Food and Drug Administration of this Department.

and extracted with ether. The ether extracted only a very small quantity of amorphous material from which nothing definite could be isolated.

The sodium hydroxide extract was made acid with hydrochloric acid and extracted with ether. After removal of the ether a dark-brown tarry product was obtained. Yield, 3 grams. This material was distilled under reduced pressure (3 mm.), and a distillate amounting to 1.0 gram was obtained. This distillate, on standing, deposited crystals, which were filtered off and recrystallized from a mixture of benzene and ligroin. Judged by their m.p., these crystals appeared to be identical with the substance melting at 122° C. (corrected) isolated from fraction K of the oil obtained on the distillation of alkali lignin from corn cobs in a reduced atmosphere of carbon dioxide (8).

From the steam-nonvolatile neutral fraction no definite substance was isolated.

SUMMARY

Alkali lignin from corn cobs was distilled with selenium in an atmosphere of nitrogen. From the oil obtained guaiacol and 1-n.-propyl-3-methoxy-4-hydroxybenzene were isolated and identified. The neutral fraction of the oil consisted of a mixture of selenium-containing organic substances. There was also isolated a crystalline substance melting at 122° C. (corr.), which appeared to be identical with the one previously isolated from the oil obtained in the dry distillation of lignin from corn cobs. No hydrocarbon that would throw light on the structure of lignin was isolated from the oily distillate.

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APPLICATION OF THE SCALES METHOD TO DETERMINATION OF SUGAR IN PLANT JUICES AND TISSUES

By WALLACE R. ROY, *Assistant Biochemist*, and AUSKER E. HUGHES, *Agent*,* (Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture)

A simple and rapid method for the accurate determination of sugars is of distinct advantage in conducting investigations on plant nutrition. With the large number of samples frequently involved in this work such a method would facilitate making the analyses directly on the fresh juices and tissues and would eliminate preservation in alcohol, which is necessary when the materials are to be stored (3). Many of the rapid methods for determining reducing sugars were developed for use in human physiological research; and to only limited extent have such methods been adapted to analyses of plant tissues.

Scales in 1915 (5) devised an accurate and rapid method, but it has some undesirable features. He showed that an accurate volumetric determination of the cuprous oxide produced by a sugar could be made on the original solution, thus eliminating filtration of the cuprous oxide and the subsequent copper determination required by previous methods. Later Clark (1) published a modification of this method. In 1919 Scales (6) reported a method that was subsequently recommended (2) for adoption by the Association of Official Agricultural Chemists, and it is now included as tentative in *Methods of Analysis, A. O. A. C.* In this method aliquots containing small amounts of reducing sugar are boiled for 3 minutes in a solution of alkaline copper sulfate, and the precipitated cuprous oxide is determined by iodometric titration. The entire procedure is carried out in a single flask.

After careful study this method was adapted to work on citrus and other subtropical fruits, because of its rapidity, ease of handling, and accuracy. Another advantage is that accurate determinations can be made with very small samples. The adaptability of the method to plant physiological studies, a comparison with the generally accepted Munson and Walker method, and a statistical study of the degree of accuracy obtainable when a slightly modified procedure is carried out under carefully controlled conditions are discussed in this paper.

METHOD

REAGENTS

The reagents used (which should be the purest obtainable) are those described in *Methods of Analysis, O.O.A.C.*, 1935, 478, **34**, with minor modifications.

* The work reported was done in connection with cooperative studies by the Division of Soil Fertility Investigations and the Division of Fruit and Vegetable Crops and Diseases of the Bureau of Plant Industry, Orlando, Florida. Credit is due Dr. H. P. Traub, Principal Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, for constructive criticism during the progress of the work, and to Dr. J. J. Skinner, Senior Biochemist, Division of Soil Fertility Investigations, Bureau of Plant Industry.

(a) *Benedict's solution.*—

$$\left.\begin{array}{l}\text{16 grams of copper sulfate } (CuSO_4 \cdot 5H_2O) \\ \text{150 ml. of water}\end{array}\right\} A$$

$$\left.\begin{array}{l}\text{15 grams of sodium citrate } (Na_3C_6H_5O_7 \cdot 2H_2O) \\ \text{130 grams of sodium carbonate, anhydrous } (Na_2CO_3) \\ \text{10 grams of sodium bicarbonate } (NaHCO_3) \\ \text{650 ml. of water}\end{array}\right\} B$$

After the constituents were thoroughly dissolved, both solutions were brought to the boiling point and solution A was slowly poured into solution B with stirring. The final solution was allowed to cool, then filtered, and the volume was made to 1 liter. (As this solution may be kept for considerable time without noticeable change, it is recommended that several liters be made at one time.)

(b) *Sodium thiosulfate solution.*—0.045 *N*. This solution was made to approximate strength at least 2 weeks before the final standardization was made. (It may be standardized against 0.05 *N* potassium dichromate and preserved in glass-stoppered bottles for several months without appreciable change in normality.)

(c) *Iodine solution.*—Approximately 0.045 *N*. Frequent blanks were made during a series of analyses, and since the strength of this solution should be slightly greater than that of the thiosulfate, 5.4 grams of iodine was dissolved in 10 ml. of water in which 9 6 grams of potassium iodide had been dissolved. When all the iodine was in solution it was made to 1 liter.

(d) *Acetic acid solution.*—24 ml. glacial acetic acid per liter.

(e) *Hydrochloric acid solution.*—60 ml. of concentrated hydrochloric acid (sp. gr. 1.19) per liter.

(f) *Starch solution.*—2.5 grams of soluble starch per liter. This solution is best prepared by stirring the weighed amount of starch into 20 ml. of water and pouring this suspension into 980 ml. of boiling water. Clear starch solutions are essential in obtaining definite end points. The solution may be prepared once each week and stored in glass-stoppered bottles without a preservative.

PROCEDURE

Ten ml. or less of the sample containing not more than 20 mg. of reducing sugar was transferred to a 250 ml. Erlenmeyer flask; 20 ml. of Benedict's solution was added, and if less than 10 ml. of sample was used the final volume was made to 30 ml. with distilled water. This volume was kept constant in all determinations. The flask was covered with a two-holed rubber stopper and placed on an electric hot plate (or flame). The source of heat was regulated to bring the solution to the boiling point in 2 minutes, and the solution was boiled exactly 3 minutes. The flask was removed and cooled by immersing in a bath of circulating tap water. (Agitation of the solution while cooling is to be avoided. If the proper water bath is provided the solution should attain room temperature in 2–3 minutes.) The rubber stopper was removed and 100 ml. of the acetic acid was added from a fast-delivering pipet; 25 ml. of the iodine solution was then accurately delivered into the solution in the flask, followed by 25 ml. of the hydrochloric acid. The solution was agitated for at least 1 minute, and the excess iodine was titrated with the sodium thiosulfate, starch being used as an indicator. In making the titration, the thiosulfate solution was run into the flask until a light sea green color was obtained. At this point 2 ml. of starch solution was added, and the titration was continued until the darker blue color faded away, leaving the light blue tint of the copper solution. The end point was definite and permanent. The volume of the sodium thiosulfate used in the titration was subtracted from a previously determined blank. The difference represents the amount of iodine acted upon by the cuprous oxide, which is proportional

to the amount of reducing sugar in the sample. The following equivalents were used directly in calculating dextrose and sucrose:

1 ml. 0.045 *N* sodium thiosulfate = 1.33 mg. dextrose.
1 ml. 0.045 *N* sodium thiosulfate = 1.26 mg. sucrose.

SODIUM THIOSULFATE EQUIVALENT OF DEXTROSE AND SUCROSE

Numerous concentrations of dextrose and sucrose were prepared from the purest forms of these substances available. These concentrations (1–30 mg.,) were subjected to the procedure described. The results are shown

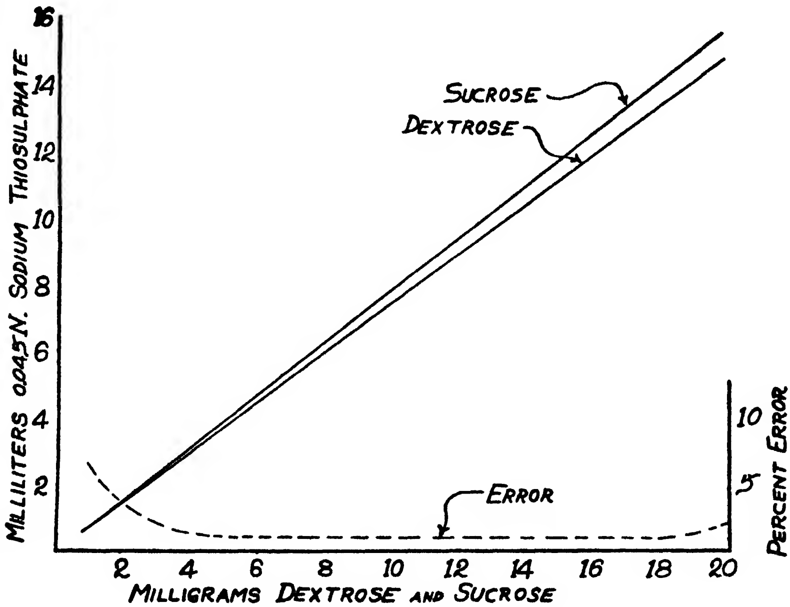

FIG. 1.—REGRESSION LINES FOR DEXTROSE AND SUCROSE WITH RELATION TO PER CENT ERROR OVER THE RANGE COVERED.

graphically in Fig. 1. The highly significant positive correlation coefficients (r) of $+0.9691 \pm .008$ for dextrose, and $+0.9978 \pm .0005$ for sucrose, were found between the milliliters of sodium thiosulfate required and the amounts of dextrose and sucrose, respectively, in the prepared solutions of the range already indicated. Since the correlations were undoubtedly linear, the straight line regression coefficients, ml. 0.045 *N* sodium thiosulfate on mg. dextrose and sucrose, respectively, were determined and the regression established by solving the regression equations for the highest and lowest determinations included in the calculations. The lines thus established statistically by the rapid machine methods of Wallace and Snedecor (8) and Treloar (7) show the same equivalents as those given previously in the method.

Six individual determinations were made with the dextrose equivalent on the six different concentrations of dextrose covering the entire range. From these results the percentage error of the amount estimated was calculated. It is plotted in Fig. 1. This curve shows that samples containing less than 4 mg. of dextrose or sucrose are subject to considerable error; above 4 mg. and up to 18 mg. the error is quite constant and small, being less than 1 per cent, and above 18 mg. the error again increases. Since the range over which the greatest accuracy will be attained is between the titration figures of 4 mg. and 17 mg., the amount of plant juice or tissue in the sample should be regulated accordingly, or solutions that are too concentrated can be diluted to the desired strengths.

Below 4 mg. of dextrose the amount of sodium thiosulfate required is so small that considerable error is introduced by using burets graduated to 0.1 ml. One drop in excess produces a decided error. Greater accuracy may be attained with a micro buret or by using a thiosulfate solution of lower normality. Above 18 mg. of dextrose the Benedict's solution is so nearly exhausted, as indicated by the lack of blue color in the solution, that it is doubtful whether the reaction ever reaches completion. If samples containing more than 18 mg. of dextrose are to be used, greater accuracy may be obtained by introducing a larger volume of Benedict's solution. If this is done, however, it is necessary to restandardize the method for these conditions.

DETERMINATION OF BLANK

Clark (1) suggests making a blank titration of thiosulfate against iodine when all the reagents except sugar are present. Scales (6) recommends a blank determination made by the titration of iodine with thiosulfate in the absence of the other reagents. He explains that with all the reagents present, but no sugar, a small amount of hydriodic acid is formed, which causes slightly low results, but in the presence of sugar all the iodine unites with the copper. It is probable that sugar only partially inhibits this reaction. Possibly a blank somewhere between that of Clark and that of Scales would be more consistently accurate. A blank can readily be established by making an initial titration of the iodine with the thiosulfate solution. This "temporary" blank is employed in making several determinations with known amounts of dextrose. The values are plotted graphically by using the milliliters of thiosulfate required as ordinates and the milligrams of dextrose as abscissas. A straight line is drawn through these points and extended to the axis. The amount of thiosulfate in excess of that required to reach the intersection should be subtracted from the temporary blank. The permanent blank thus obtained should be used in all determinations. When a fresh stock of Benedict's solution or thiosulfate solution is introduced, it is advisable to determine a new blank. Large quantities of each of these solutions should be made in order to eliminate frequent blank determinations.

THE EFFECT OF TIME OF BOILING UPON THE COPPER REDUCTION

Clark (1) has shown the importance of standardizing the time of heating. Varying amounts of invert sugar and of solution were subjected to different periods of heating. With the conditions under which Clark was working, 3–4 minutes was required to bring the solution to boiling, and actual boiling was maintained for 2 minutes. Heating longer than 4.5 minutes produced a gradual decrease in the amounts of cuprous oxide present.

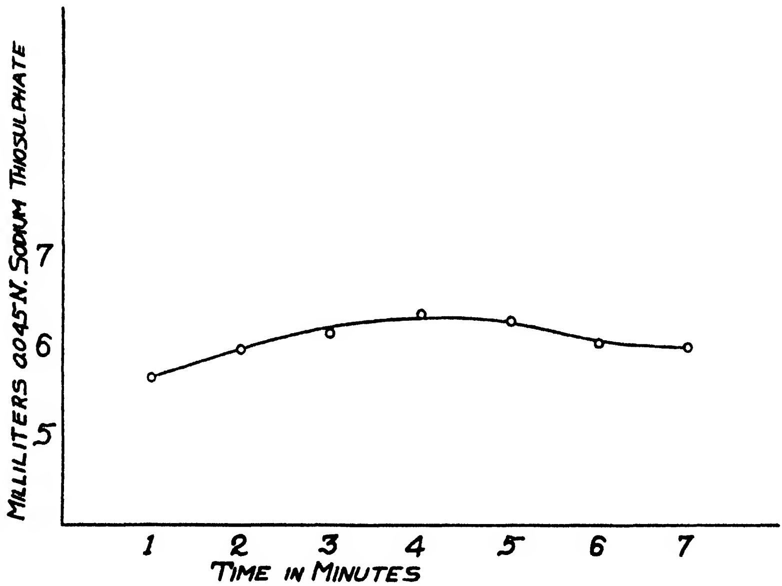

FIG. 2.—EFFECT OF PERIOD OF BOILING ON THE VOLUME OF 0.045 N SODIUM THIOSULFATE REQUIRED BY 8 MG. OF DEXTROSE WHERE 2 MINUTES IS THE TIME REQUIRED TO ATTAIN THE BOILING POINT.

The hot plate used by Scales was so regulated that 5 minutes was required to bring the 30 ml. of solution to boiling. Boiling was then continued for exactly 3 minutes. As precipitation of cuprous oxide begins to take place at temperatures near the boiling point it appears that the time required to reach this point is negligible in comparison with the time of actual boiling. The source of heat should be regular and uniform. The flasks used should be uniform in thickness and possess equal contact areas. Comparable results can not be obtained when different volumes are used due to the variation in time required to bring the solutions to boiling. From the curve plotted in Fig. 2, where two minutes was required to bring the solution to boiling, it is evident that the greatest amount of cuprous oxide was obtained between 3 and 4 minutes. For boiling periods

in excess of 4 minutes the amount decreased, but little difference was noted between 3 and 5 minutes of boiling. Three minutes of actual boiling is sufficient.

PREVENTION OF BACK OXIDATION OF CUPROUS OXIDE

After the precipitation of cuprous oxide is complete and the flask has been removed from the hot plate and placed in the water bath a condensation of the water vapor within the flask causes a reduction in pressure. Air is drawn into the flask, and if the solution is agitated a portion of the cuprous oxide may be brought in contact with the oxygen of the air and oxidized to the cupric form, thereby causing low results. In a series of experiments in which the flask was agitated during the cooling period, it was found that the differential titrations were from 0.2 to 0.4 ml. less than duplicate determinations wherein the flask was held immobile. This might be explained by the fact that when the flask is allowed to remain perfectly still the greater portion of the oxide is beneath the surface of the solution and protected from the air.

After the solution has been cooled the dilute acetic acid should be delivered into the flask from a fast delivering pipet, the tip of which is held near the surface of the solution, to prevent the entrance of mechanically occluded air. Carbon dioxide liberated by the acetic acid protects the cuprous oxide through the remainder of the analysis.

DETERMINATION OF DEXTROSE AND SUCROSE IN MIXTURES

Various concentrations of equal mixtures of dextrose and sucrose were prepared, and dextrose was determined directly. Equal volumes, usually

TABLE 1.—*Effect of dextrose and sucrose upon the reduction of copper present in mixtures*

SUGARS PRESENT IN MIXTURE		0.045 *N* THIOSULFATE REQUIRED BY DEXTROSE	DEXTROSE FOUND	0.045 *N* THIOSULFATE REQUIRED AFTER INVERSION	0.045 *N* THIOSULFATE REQUIRED BY SUCROSE	SUCROSE FOUND	TOTAL SUGARS FOUND
DEXTROSE	SUCROSE						
mg.	*mg.*	*ml.*	*mg.*	*ml.*	*ml.*	*mg.*	*mg.*
1	1	0.76	1.01	1.55	0.79	1.00	2.01
2	2	1.51	2.01	3.10	1.59	2.02	4 03
3	3	2.18	2.90	4.61	2.43	3.09	5.99
4	4	3.05	4.06	6.15	3.10	3.94	8.00
5	5	3.68	4.90	7.80	4.12	5.23	10.13
8	8	6.05	8.05	12.37	6.32	8.03	15.95
10	10	7.60	10.11	15.30	7.70	9.78	19.89
12	12	8.90	11.84	18.51*	9.61	12.20	24.04
15	15	11.15	14.83	23.20*	12.05	15.30	30.13
20	20	14.80	19.68	30.60*	15.80	20.07	39.73

* Determinations made on ½ volume.

25 ml., were then subjected to hydrolysis in the presence of hydrochloric acid (sp. gr. 1.1029). After complete inversion the acid was exactly neutralized with sodium hydroxide of equal strength. The solution was then made to a known volume, and the reducing sugars were again determined upon aliquots. Sucrose was calculated from the final titration. The results are given in Table 1. They are comparable and in close agreement. Solutions containing both dextrose and sucrose will have a much higher cuprous oxide content after inversion, so it is imperative to take smaller samples for the final determination.

DETERMINATION OF DEXTROSE AND SUCROSE IN PLANT JUICES AND PULPS

Types of fruit that lend themselves to juice extraction by means of pressure are relatively easier to study than are those that do not. Leaf tissues, stem and root tissues, and various fruit products must be subjected to some type of extraction. The most widely used method for extraction from fruit tissue is that by boiling water. The fruit flesh is ground through a food chopper and mixed thoroughly. A representative sample, usually 100 grams, is placed in a liter beaker, and 450 ml. of water is added along with 1.0 gram of calcium carbonate to effect neutralization of any acid present. The mixture is boiled for 1 hour, cooled, made to 500 ml. volume, and filtered. When leafy or woody tissues are being analyzed one of the alcohol extraction methods described by Loomis and Shull (3) is recommended. After the alcohol has been evaporated off, this extract may be treated in the same manner as any fruit juice.

When determinations of both sugars are being made, clarification (4) with neutral lead acetate is imperative. After clarification, an aliquot is taken for the determination of reducing substances. If orange juice or similar substances are to be analyzed an aliquot representing 0.5 gram of the juice is desirable. Half of this amount is sufficient for the final determination after inversion. A practice that has given satisfactory results is as follows: 25 grams of juice or extract is clarified and made to a volume of 250 ml. A 5 ml. aliquot of this solution is used for the determination of dextrose. A 25 ml. aliquot of the same solution is also withdrawn and hydrolyzed in the presence of 10 ml. of hydrochloric acid (sp. gr. 1.1029). The slow inversion method, which requires the solution to stand overnight at a temperature of 25° C. or above, is used. After complete inversion is attained, the acid required for hydrolysis is exactly neutralized with 10 ml. of sodium hydroxide solution of the same strength, and the volume is completed to 50 ml. Aliquots of 5 ml., representing 0.25 gram of the original juice or extract, are taken for the final titration.

The following formulas may be used in calculating dextrose and sucrose from the titrations:

TABLE 2.—*Modified Scales method compared with Munson and Walker method*

TYPE OF SAMPLE	NUMBER OF VARIATES	REDUCING SUBSTANCES EXPRESSED AS DEXTROSE				NON-REDUCING SUBSTANCES EXPRESSED AS SUCROSE			
		MUNSON-WALKER		SCALES		MUNSON-WALKER		SCALES	
		MEAN ± P.E.	C.V	MEAN ± P.E.	C.V.	MEAN ± P.E.	C.V.	MEAN ± P.E.	C.V.
Dextrose 60 mg.	12	60.2 ± .205	1.75	59.99 ± .104	.89	—	—	—	—
Mixture Dextrose 75 mg. Sucrose 75 mg.	10	75.37 ± .07	.44	75 32 ± .08	.47	75.23 ± .06	.35	74.63 ± .12	.77
Orange Juice	10	3.23 ± .004	.52	3.27 ± .004	56	4.33 ± 008	.79	4.40 ± .011	1.18

* P.E. = Probable error. C.V. = Coefficient of variability.

$$\frac{D_t \times 1.33 \times 100}{S} = \text{per cent reducing substances as dextrose, and}$$

$$\frac{(S_t - \frac{1}{2}D_t)1.27 \times 100}{S} = \text{per cent inverted sugars as sucrose, where}$$

D_t = ml. 0.045 N thiosulfate required in dextrose titration,
S_t = ml. 0.045 N thiosulfate required in sucrose titration, and
S = Wt. of sample (mg.).

Results may be reported as total reducing substances and total sugars, as these terms express more correctly the constituents being determined.

COMPARISON WITH ESTABLISHED STANDARD METHODS

A statistical comparison was made of the Scales method as modified herein and the generally accepted method of Munson and Walker. This study includes twelve determinations on dextrose alone, ten determinations of mixtures of dextrose and sucrose, and ten determinations of the reducing and non-reducing sugars in a representative sample of orange juice.

The data obtained were subjected to statistical analysis, and the probable errors together with the respective coefficients of variability are given in Table 2. These results show a close agreement between the two methods. When pure sugar solutions were analyzed it was found that the probable error was very low in each case, making the mean very significant. The standard deviation, expressed on a percentage basis (the coefficient of variability, C. V.), is less than 1 per cent in all cases except one, where it is 1.75 per cent. Compared on the basis of sugar determinations in orange juice, the two methods again give almost identical results, the coefficients of variability ranging from 0.52 to 1.18.

Closer agreement was obtained in the direct determination of reducing sugars than in the determination of invert sugars where reducing sugars were also present. The differences are largely due to accumulative errors unavoidably introduced during the process of inversion. However, the means are highly significant and practically identical for both methods. When employed to determine sugars in the optimum range of concentration, the Scales method has an error of 1 per cent or less of the amount estimated. This small error is in the minimum range for any volumetric method.

SUMMARY

The Scales method for the determination of reducing and non-reducing sugars was studied in comparison with a standard method from the standpoint of its adaptability to the analysis of plant juices and tissues. The method was verified statistically and modified in certain particulars.

1. The sodium thiosulfate-sugar equivalent was determined by means of regression lines of volume of sodium thiosulfate on milligrams of dextrose and sucrose, covering the range considered. A statistical study of these ratios shows them to be linear.

2. A method for the determination of the blank, based on the fact that linear regression lines were obtained, is presented.
3. The effect of period of boiling on the amount of sugar recovered was studied. The optimum time of actual boiling was found to be 3 minutes.
4. A source of error due to back oxidation was controlled during cooling by minimum agitation of the flask in which the determination was being made.
5. A statistical study of the error in the amount of sugar, estimated at various concentrations and covering the entire range of the method, shows that the method is reliable between the limits of 4–18 mg. of sugar.
6. A statistical comparison of the Scales method as modified and the standard method of Munson and Walker, utilizing both pure sugar solutions and fruit juices, shows the same degree of accuracy for both methods.

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AN IMPROVED TECHNIC IN THE TOLUENE DISTILLATION METHOD FOR THE DETERMINATION OF MOISTURE IN FOODSTUFFS

By J. A. de Loureiro (Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.)

The Bidwell-Sterling[1] method is particularly applicable to the estimation of the water content of biological substrates. It involves heating the material in toluene, which has a boiling point only slightly higher than that of water, and collecting the distilled water under toluene, with which water is not miscible. The principle is sound and simple, but the apparatus devised by the authors does not work satisfactorily, in that droplets of water usually stick to the walls of the condenser and collecting tube, causing an appreciable error. Previous cleaning of the glass parts with chromo-sulfuric solution does not obviate this condition, particularly if the material contains traces of volatile fatty acids.

Calderwood and Piechowsky[2] have recently discussed the importance

[1] *This Journal*, **8**, 295 (1925).
[2] *Ind. Eng. Chem. Anal. Ed.*, **29**, 520 (1938).

of removing this condensed water adhering to the inner wall of the condenser tube, and suggested a scheme for this purpose. The writer found that an ideal condition can be obtained most easily by modifying the whole apparatus so as to avoid entirely any adhesion of water outside of the measuring device, instead of trying to drain down the water deposited on the condenser wall.

The reason for the lack of complete drainage in the graduated part of the tube in the Bidwell-Sterling apparatus is twofold. The first is mechanical. In an ordinary reflux condenser, in which the vapors travel upwards, toluene, the less volatile liquid, condenses below the water, and there is none left to sweep down the condensed droplets of water. The other reason is physico-chemical. At neutral or acid reaction water has more affinity for the glass than has toluene, hence it tends to deposit along the walls of the apparatus as stubbornly adherent droplets. The condition is reversed when the reaction is strongly alkaline. Then the glass is easily moistened by toluene, and gravity will cause even the smallest drops of water to slide along the walls.

These two faults can obviously be corrected by changing the direction of the flow of vapors and by keeping the surface of the glass alkaline.

The first prerequisite is fulfilled by the apparatus shown in Fig. 1. It consists of a 300 ml. Erlenmeyer distilling flask, fitted with a ground-in delivery tube that carries the vapors into a one-piece condenser-collecting-measuring tube. This tube has an upper section 20–25 cm. long and 2 cm. in diameter, and a lower section 16–18 cm. long and 0.8 cm. in diameter, which is graduated and functions as the measuring device. The upper part, cooled and kept in place by means of a cold water jacket, functions as a condenser.

The collecting tube is first filled with toluene to 2 or 3 cm. above the graduated part, and the end of the outlet tube from the distilling flask is immersed below the surface of the toluene. As both toluene and water have relatively high boiling points, the cooled toluene contained in the tube is sufficient to retain all vapors if the distillation is conducted at a moderate rate. An electric hot plate at the "low" switch is generally satisfactory. There is no loss by evaporation, and as the vapors flow in the same direction as the condensate all the water is drained down. The distillation is generally complete before the collecting tube is filled. Should this not be the case, the heating should be discontinued for a moment to allow the excess toluene to be sucked back into the flask.

In order to avoid the deposition of water on the walls of the collecting tube, it is necessary to make the surface of the glass alkaline. This is done by rinsing the tube with 5 per cent potassium hydroxide solution after it has been cleaned with chromo-sulfuric solution and rinsed with water, and while still wet. The alkaline solution is allowed to drain for 5–10 minutes, and then the necessary quantity of toluene is run into the tube.

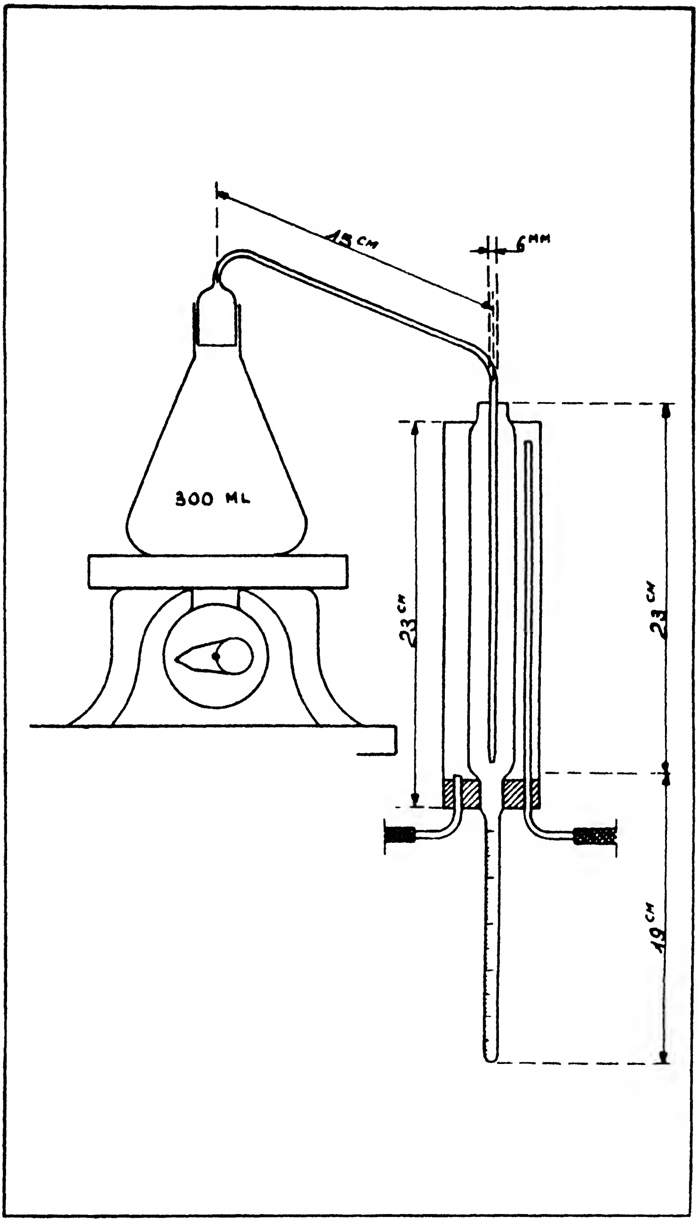

FIG. 1

These precautions are sufficient if the material is perfectly neutral, but if it contains even traces of volatile fatty acids, as is the case with certain animal tissues, these acids will neutralize the small quantity of alkali

deposited on the glass, and water will begin to stick to the walls. To prevent the distillation of these fatty acids, a mild alkaline salt should be added to the material. Disodium phosphate was found to be satisfactory. The alkalinity of this salt is so slight that there is no danger of splitting neutral fat, and as large quantities of free fatty acids never exist in fresh tissues the volume of water produced by the neutralization of the amounts normally present is negligible. On the other hand the salt itself does not retain appreciable quantities of water at the boiling point of toluene. When 5 ml. of water is added to 14.2 grams (0.1 *M*) of anhydrous disodium phosphate, 4.92–4.94 ml. of water is recovered in the distillate. This loss, too small to represent any water of crystallization, may be due to occluded water between the crystals, because dipotassium phosphate, which forms anhydrous crystals, retains more water than does the sodium salt under the same conditions. It may be concluded, therefore, that the error depending on the presence of 1 gram or less of disodium phosphate dispersed into the material is negligible.

To summarize, satisfactory results are obtained with Bidwell and Sterling's so-called "direct method for water estimation" under the following conditions:

(1) An apparatus allowing a flow of liquid in the same direction as the flow of vapors must be used.

(2) The collecting tube must be rinsed with a strongly alkaline solution before the estimation is begun.

(3) Liberation of volatile fatty acids must be prevented by the addition of a small quantity of anhydrous disodium phosphate.

ANALYSES OF SOME INDIAN FOOD PLANTS*

By E. Yanovsky and R. M. Kingsbury

More interest in the diet of the North American aborigines has been shown by botanists and ethnologists than by chemists or specialists in nutrition. There is voluminous ethnobotanical literature dealing with the food, medicinal, and other useful plants of the Indians. A summary of this literature dealing with vegetable foods, prepared by Yanovsky (20), describes the use of over 1,100 species of food plants. It is safe to say, however, that barring cultivated crops, not much more than one per cent of these plants has been even approximately analyzed. Accordingly, the data presented in the present paper, although some of the analyses are not complete, may be of considerable interest to plant chemists and students of nutrition.

* Contribution No. 138 from the Carbohydrate Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

TABLE 1.—*Analyses of some cryptograms**
(Results expressed in percentage)

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
1	9.4	0.3	0.0	22.5†	46.8	—	—	—	—	*Alectoria jubata* (L) Ach.
	—	0.3	0.0	24.8†	51.7	1.6	5.5	4.3	1.6	A lichen of the Fam. *Usneaceae*, Utah
1a	9.6	0.0	0.0	15.8†	32.0	—	—	—	—	Ditto. Oregon
	—	0.0	0.0	17.5†	35.4	—	—	—	—	
2	9.3	4.6	0.0	0.0	9.9	—	—	—	—	*Pteridium aquilinum pubescens Underw.*—Bracken
	—	5.1	0.0	0.0	10.9	0.9	3.1	26.6	7.3	A fern of the Fam. *Polypodiaceae*, Utah.
2a	7.8	11.1	0.0	12.3	16.0	—	—	—	—	Ditto. Alaska
	—	12.0	0.0	13.3	17.4	—	—	—	—	
2b	8.4	6.2	3.9	5.0	19.9	—	—	—	—	Ditto. California
	—	6.8	4.3	5.5	21.7	—	—	—	—	

* The second line of figures for each plant in this and following tables represents values determined or calculated on dry basis.
† Starch in case of *Alectoria jubata* is "lichenin."

TABLE 2.—*Analyses of coontie roots*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH*	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	ASH	SPECIES AND ORIGIN
3	62.1	0.0	1.6	18 8	7.2	—	—	—	*Zamia floridana A. DC.*—Coontie
	—	0.0	4.2	49.6	19.0	0.4	10.3	2.6	Fam. *Cycadaceae*, Florida
3a	63.7	0.6	2.1	19.4	4.3	—	—	—	Ditto
	—	1 7	5.8	53.4	11.8	—	3.4	3.7	

* These figures for starch are somewhat higher than the one (37.8%) obtained by Clevenger (1)

TABLE 3.—*Plants of the family Pinaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
4	16.1	3.3	0.0	0.0	11.0					*Juniperus californica* Carr.—California Juniper
	—	3.9	0.0	0.0	13.1					Berries from California
4a	21.2	0.5	0.8	0.0	11.0					Ditto
	—	0.6	1.0	0.0	14.0					
5	25.3	19.6	0.0	0.0	9.1					*Juniperus occidentalis* Hook.—Western Juniper
	—	26.2	0.0	0.0	12.2					Berries from California
6	23.1	6.5	0.0	0.0	9.3					*Juniperus utahensis* (Engelm.) Lemmon—Utah Juniper
	—	8.5	0.0	0.0	12.1					Berries from Utah
6a	10.8	6.9	0.5	0.0	12.0					Ditto
	—	7.7	0.6	0.0	13.5					
7	6.5	0.8	0.4	0.0	26.1					*Pinus contorta* Dougl.—Shore Pine
	—	0.9	0.4	0.0	27.9					Cambium from Oregon
7a	6.5	1.0	2.0	0.0	22.2					Ditto. Alaska
	—	1.1	2.1	0.0	23.7	—	2.4	—	2.3	
8	25.1	2.4	3.7	7.2	4.2	35.2	—	—	—	*Pinus edulis* Engelm.—Nut Pine
	—	3.2	4.9	9.6	5.6	47.1	—	—	—	Nuts from Utah
8a	3.0	0.0	7.6	5.2	4.2	58.1				Ditto. Colorado
	—	0.0	7.8	5.3	4.3	59.9				
9	9.0	1.8	1.4	0.0	9.5	—	—	41.8	—	*Thuja plicata D.* Don.—Giant Arborvitae
	—	2.0	1.5	0.0	10.4	—	—	45.9	—	Whole bark from Alaska
9a	5.8	1.5	3.7	0.0	11.6					Ditto. Cambium from Oregon
	—	1.6	3.9	0.0	12.3					

TABLE 4.—*Plants of the families Poaceae and Cyperaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	ASH	SPECIES AND ORIGIN
10	10.0	*Total* 5.8		18.7	—	—	—	2.6	*Oryzopsis hymenoides* (Roem.and Schult.) Ricker. Indian Rice Grass. Fam. *Poaceae*. Seeds from New Mexico
	—	6.4		20.1	—	—	—	2.9	
11	4.7	2.4	4.9	0.0	17.0	—	—	—	*Phragmites communis* Trin.—Common Reed. Fam. *Poaceae*. Stems, leaves, and seeds from Nevada
	—	2.5	5.1	0.0	17.8	—	0.5	14.4	
12	8.0	0.0	2.3	61.1	4.9	—	—	—	*Zizania aquatica* L.—Annual Wildrice. Fam. *Poaceae*. Seeds from Minnesota
	—	0.0	2.5	66.4	5.3	1.0	1.8	1.7	
13	5.1	2.9	5.0	5.2	18.8	—	—	—	*Scirpus validus* Vahl.—Great Bulrush. Fam. *Cyperaceae*. Roots from Nebraska
	—	3.1	5.3	5.5	19.8	—	0.4	15.5	

TABLE 5.—*Plants of the family Liliaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR*	STARCH	HEMI-CELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
14	69.8	1.7	18.1	0.0	3.2	—	—	—	—	*Allium nuttallii* S. Wats.
	—	5.6	59.9	0.0	10.6	—	5.4	—	2.1	Bulbs from Nebraska
14a	58.9	0.7	20.7	0.0	4.2	—	—	—	—	Ditto
	—	1.7	50.4	0.0	10.2	—	9.8	—	3.2	
15	77.2	2.4	3.6	4.0	5.9	—	—	—	—	*Calochortus luteus* Dougl.—Yellow Mariposa
	—	10.5	15.8	17.5	25.9	0.6	9.1	—	6.2	Bulbs from Utah
16	20.8	9.7	7.3	35.5	7.8	—	—	—	—	*Erythronium grandiflorum* Pursh.—Glacier lily
	—	12.2	9.2	44.8	9.8	—	5.3	—	3.6	Bulbs from Montana
17	9.0	10.4	10.1	26.4	3.4	—	—	—	—	*Fritillaria kamschatensis* (L.) Ker.
	—	11.6	11.2	29.3	3.8	—	—	5.7	—	Bulbs from Alaska
18	75.5	1.1	4.9	4.9	4.5	—	—	—	—	*Fritillaria pudica* (Pursh) Spreng.
	—	4.5	20.0	20.0	18.4	—	7.8	—	4.7	Bulbs from Utah
19	43.8	1.5	14.3	17.9	7.8					*Hookera coronaria* Salisb.
	—	2.7	25.4	31.9	13.9					Bulbs from California
20	69.5	0.5	11.2	0.0	4.9					*Quamasia leichtlinii* (Baker) Coville
	—	1.6	36.6	0.0	15.8	—	11.7	—	3.2	Leichtlin camas. Bulbs from Washington
21	50.5	2.8	22.0	0.0	15.8	—	—	—	—	*Quamasia quamash* (Pursh) Coville (*Camassia esculenta*
	—	5.7	44.4	0.0	32.0	1.1	4.6	3.0	3.0	Lindl.) —Common Camas. Bulbs from Utah
21a	58.2	2.8	15.2	0.0	13.6	—	—	—	—	Ditto. Idaho
	—	6.7	36.6	0.0	32.6	0.5	—	—	1.8	
21b	66.0	3.0	11.6	0.0	6.8	—	—	—	—	Ditto. Oregon
	—	8.8	34.1	0.0	20.0	0.9	—	—	0.9	
21c	68.0	2.2	13.3	0.0	9.4	—	—	—	—	Ditto. Washington
	—	6.9	41.6	0.0	29.4	—	2.4	—	1.4	
21d	68.3	2.7	14.2	0.0	2.2	—	—	—	—	Ditto. Idaho
	—	8.5	44.8	0.0	6.9	—	—	—	2.4	
21e	66.9	1.5	15.0	0.0	5.7	—	—	—	—	Ditto. Montana
	—	4.5	45.3	0.0	17.2	—	9.4	—	—	
22	31.6	4.3	0.0	0.0	11.4	—	—	—	—	*Yucca baccata* Torr.—Banana Yucca
	—	6.3	0.0	0.0	16.7	7.6	7.0	49.7	4.6	Fruit from New Mexico
22a	8.4	5.6	3.4	0.0	11.9	—	—	—	—	Ditto
	—	6.1	3.7	0.0	13.1	—	10.2	—	4.6	

* The figures for non-reducing sugars for Allium and Camas bulbs represent inulin always found in these plants (21). Ash of 21d was analysed with the following results: P_2O_5—19.0%, CaO—12.7%, Mn_3O_4—3.2%, MgO—4.5%, $Fe_2O_3 \cdot Al_2O_3$—trace.

TABLE 6.—*Plants of the families Urticaceae, Polygonaceae, and Nyctaginaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	ASH	SPECIES AND ORIGIN
23	15.3	27.1	0.0	0.0	4.8	—	—	—	*Celtis occidentalis* L.-Hackberry*. Fam. *Urticaceae.* Berries from Nebraska
	—	32.2	0.0	0.0	5.7	—	11.6	25.9	
23a	17.4	29.6	0.0	0.0	4.6	—	—	—	Ditto. Kansas
	—	35.8	0.0	0.0	5.6	2.8	10.0	31.7	
23b	19.0	26.9	0.0	0.0	1.6				Ditto. Nebraska
	—	33.2	0.0	0.0	2 0				
23c	5.1	0.5	0.2	0.0	2.2				Ditto. Green berries from Nebraska
	—	0.5	0.2	0.0	2.3	—	—	36.7	
24 Whole	58.1 root	1.2	1.2	4.4	7 0				*Rumex hymenosepalus* Torr.—Canaigre. Fam. *Polygonaceae.* Roots from Arizona
	—	2.9	2.9	10.5	16.7	—	14.0	4.9	
24a Decorti	61.1 cated	2.0 root	2.2	6.7	5.5				Ditto. New Mexico
	—	5.1	5.7	17.2	14.1	—	—	3.6	
24a Bark	58.6	1.4	4.0	3.3	4.3				
	—	3.4	9.7	8 0	10.4	—	—	7.3	
25	90.2	0.0	0.7	0.0	1 9				*Abronia latifolia* Eschsch.—Yellow Sandverbena. Fam. *Nyctaginaceae.* Roots from Oregon
	—	0.0	7.1	0 0	19.4	—	11.3	18.8	

* Pulp and pits ground together, as used for food by various Indian tribes, were analysed in the case of hackberries Analysis of the abnormally high ash of the berries showed (22) that there is about 25% of calcium carbonate in the dry weight of the fruit.

TABLE 7.—*Plants of the family Portulacaceae*

PLANT	MOISTURE	REDUCING SUGARS	NON-REDUCING SUGARS	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
26	74.5	1.1	1.8	8.5	6.6	—	—	—	—	*Claytonia lanceolata* Pursh. Tubers from Utah
	—	4.3	7.1	33.3	25.9	0.9	7.9	—	4.0	
Decorticated Roots: 27	11.6	1.1*	4.1*	25.4*	8.2	—	—	—	—	*Lewisia rediviva* Pursh.—Bitterroot. Roots from Utah
	—	1.3	4.6	28.7	9.2	1.0	5.9	9.1	3.9	
27a	8.7	5.6	3.2	34.2	3.6					Ditto. California
	—	6.1	3.5	37.5	3.9					
27b	10.3	1.0	3.2	41.0	11.4	—	—	—	—	Ditto. Oregon
	—	1.1	3.6	45.7	12.7	—	7.0	—	4.2	
27c	11.2	0.9	4.1	42.3	9.7	—	—	—	—	Ditto. Oregon
	—	1.0	4.6	47.6	10.9	—	3.6	—	4.1	
27d	6.3	2.1	1.5	17.9	8.6	—	—	—	—	Ditto. Washington
Root	with	bark								
	—	2.2	1.6	19.1	9.2	—	—	—	8.3	

* Trimble (13) in his analysis of *Lewisia rediviva* could detect no sugar in the plant, and his value for starch (8.6%) is considerably lower than our value.

TABLE 8.—*Plants of the families Nymphaeaceae and Berberidaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	ASH	SPECIES AND ORIGIN
28	18.1	1.1	0.6	29.1	14.6	—	—	—	*Nelumbo lutea* (Willd.) Pers.—American Lotus
	—	1.3	0.7	36.8	17.8	—	13.3	5.7	Fam. *Nymphaeaceae.* Tubers from Minnesota
28a	7.6	0.0	11.0	30.2	10.0	—	—	—	Ditto. Seeds from Minnesota, shelled for analysis
	—	0.0	11.9	32.6	10.8	2.3	18.9	4.4	
29	88.8	0.4	0.4	0.0	1.5	—	—	—	*Nymphaea advena* Ait.—Spatterdock
	—	3.6	3.6	0.0	13.4	—	—	8.0	Fam. *Nymphaeaceae.* Tubers from Illinois
30	75.9	6.5	0.0	0.0	3.3				*Berberis aquifolium* Pursh.—Oregon Hollygrape
	—	27.0	0.0	0.0	13.7				Fam. *Berberidaceae.* Berries from Montana

TABLE 9.—*Plants of the family Rosaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	HEMICELLULOSE	PROTEIN	ASH	SPECIES AND ORIGIN
31	78.0	6.0	0.0	6.1			*Amelanchier alnifolia* Nutt.—Saskatoon
	—	27.3	0.0	27.7			Berries from Montana
31a	66.3	10.3	0.0	4.3			Ditto. Utah
	—	30.7	0.0	12.7			
32	63.0	11.9	0.0	7.4			*Amelanchier canadensis* (L.) Medic.—Downy Shadblow.
	—	32.2	0.0	20.0			Berries from Utah
33	6.9	3.2	0.0	14.3	—	—	*Crataegus douglasii* Lindl.—Black Hawthorn
	—	3.4	0.0	15.4	0.3	3.2	Berries from Utah
34	15.0	15.2	0.0	11.0	—	—	*Rosa nutkana* Presl.—Nutka Rose
	—	17.9	0.0	13.0	6.6	5.7	Ripe berries from Idaho
34a	66.2	0.7	0.0	8.1			Ditto. Green berries from Alaska
	—	2.1	0.0	24.0			

TABLE 10.—*Plants of the family Mimosaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	HEMICELLULOSE	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
35	5.5	2.1	20.5	13.8				*Prosopis glandulosa* Torr.—Mesquite*
	—	2.2	21.7	14.7				Pods and beans from New Mexico
35a	6.4	1.9	12.4	17.1				Ditto. California
	—	2.0	13.2	18.3				
35b	6.4	0.6	6.4	20.4	—	31.6		Ditto. Utah
	—	0.6	6.8	21.8	—	33.8		
35c	10.7	0.9	3.5	28.0	—	—	—	Ditto. Utah
	—	1.0	3.9	31.4	1.3	—	4.7	
36	6.9	0.7	19.2	17.0				*Strombocarpa odorata* (Torr. and Frem.) Torr.—
	—	0.8	21.4	18.3				Screwbean. Pods and beans from Utah
36a	11.0	1.0	18.3	14.8				Ditto. California
	—	1.1	20.6	16.6				
36b	8.2	1.7	25.2	12.0	—	—	—	Ditto. Arizona
	—	1.9	27.5	13.1	9.4	—	4.2	
36c	8.0	3.9	17.9	33.6	—	—	—	Ditto. Utah
	—	4.2	19.5	36.5	1.3	—	3.9	

* A number of analyses on mesquite fruit are given in Walton's bulletin (18).

TABLE 11.—*Plants of the family Fabaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
37	7.9	0.6	5.5	21.2	—	—	—	—	—	*Falcata comosa* (L.) Kuntze—Hogpeanut. Tubers
	—	0.7	6.0	22.1	—	—	24 5	—	5.1	from Michigan
38	8.2	1.6	12.7	14.0	24.1	—	—	—	—	*Glycine apios* L. (Apios tuberosa)—Potatobean. Under-
	—	1.7	13.8	15.3	26.3	—	17.5	—	4.7	ground fruit from Michigan
39	9.6	1.2	4.9	2.8	18.0	—	—	—	4.4	*Glycyrrhiza lepidota* Pursh.—Licorice. Rootstocks from
	—	1.3	5.4	3.1	19.9	1.1	14.5	47.6	4.9	Washington
39a	4.6	1.0	4.5	3.0	11.0	—	—	—	—	Ditto. Nebraska
	—	1.0	4.7	3.1	11.5	—	13.7	—	7.7	
39b	4.7	0.0	9.9	6.8	14.2	—	—	—	—	Ditto. Washington
	—	0.0	10.4	7.1	14.9	—	15.0	—	11.4	
40	24.6	2.8	7 5	17.5	12.8	—	—	—	—	*Psoralea esculenta* Pursh.—Indian Breadroot. Roots
	—	3.7	9 9	23.2	17.0	1.0	7.7	—	2.9	from Nebraska
40a	43.9	0.9	7.4	17.1	12.0	—	—	—	—	Ditto. Nebraska
	—	1.6	13 2	30 5	21.4	—	13.1	—	3.1	

TABLE 12.—*Composition of ash of glycyrrhiza rootstocks*

	G. L., ASH=4.4%		G. G., ASH=6.2%	
	ASH	PLANT	ASH	PLANT
	per cent	*per cent*	*per cent*	*per cent*
K_2O	12.9	0.6	18.4	1.1
P_2O_5	8.1	0.4	4.0	0.2
CaO	40.2	1.8	22.4	1.4
MgO	4.9	0.2	7.1	0.4
Mn_3O_4	0.025	0.001	0.052	0.003
Fe_2O_3 (mostly) $+Al_2O_3$	6.1	0.3	11.3	0.7

Only 66 species, or about 6 per cent of the plants enumerated by Yanovsky are represented in this study. These species, however, are distributed among 28 families, or about 23 per cent of those described. Among the plants analyzed are some that were very popular as foods among the Indians, e.g., camas roots, yampa, bitterroot, and others; a certain number of them were admittedly poor foods, e.g., cambium of shore pine, hemlock, and others; and most of the plants ranged between these two extremes. It is believed that the following data will give a fair picture of the Indian vegetable diet.

ANALYTICAL METHODS

Official methods (10) were used in the various determinations. Moisture was determined by preliminary drying in vacuo at 60°–70° C., and then by drying in an oven at 105°–110° C. to constant weight. Samples for sugar determination were prepared as outlined on page 341 of *Methods of Analysis* (10), and the sugars were determined by the Munson and Walker method, reducing sugars as glucose and non-reducing sugars as sucrose. A polariscopic reading was taken on all the solutions prepared for sugar determination, both before and after inversion. In many cases this practice will give an approximate idea as to the nature of the sugar in the plant. Thus, positive rotation of sucrose will change to negative rotation of invert sugar. By this method the presence of inulin was discovered in several plants (negative rotation changed after inversion to a higher negative rotation).

Starch in the plants was determined by both acid and diastase methods, and the difference between the two values is recorded as hemicellulose.

For licorice plants (Table 11, 39, 39a, 39b) crude glycyrrhizin was determined by extraction with alcohol, evaporating the alcohol, precipitating with dilute sulfuric acid, redissolving in dilute ammonia, and reprecipitating with sulfuric acid (6, 8, 17). The results (on dry basis) were as follows: 7.7 per cent, 14.8 per cent and 10.2 per cent.

None of the three samples of licorice examined possessed the sweet taste characteristic of the officinal plant (*Glycyrrhiza glabra* L.).

TABLE 13.—*Plants of the families Empetraceae, Aesculaceae, Cactaceae, and Elaeagnaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	ASH	SPECIES AND ORIGIN
41	91.3	2.5	0.0	0.0	2.0				*Empetrum nigrum* L.—Crowberry. Fam. ***Empetraceae.*** Berries
	—	28.7	0.0	0.0	23.0				from Alaska
42	71.0	0.7	2.7	3.3	1.4	—	—	—	*Aesculus californica* (Spach) Nutt.—California Buckeye. Fam.
	—	2.4	9.3	11.4	4.8	0.8	23.1	5.3	*Aesculaceae.* Nuts from California
42a	67.0	0.3	4.2	6.3	8.4	—			Ditto. California
	—	0.9	12.7	19.1	26.5	1.5			
43	80.3	0.3	0.2	0.0	1.9	—	—		*Ariocarpus fissuratus* (Engelm.) K. Schum.—Living-rock. Fam.
	—	1.5	1.0	0.0	9.6	—	4.1		*Cactaceae.* Plant from California
44	84.5	0.2	0.2	0.0	1.1	—	—	—	*Lophophora williamsii* (Lemaire) Coult.—Peyote. Fam. *Cactaceae.*
	—	1.3	1.3	0.0	7.1	—	15.6	25 7	Plant from Texas
45	70.8	12.5	0.0	0.0	1.8				*Lepargyrea argentea* (Pursh.) Greene—Silver Buffaloberry. Fam.
	—	42.8	0.0	0.0	6.2				*Elaeagnaceae.* Berries from Utah
46	74.1	11.6	0.0	0.0	1.1				*Lepargyrea canadensis* (L.) Greene—Russet Buffaloberry. Fam.
	—	44.8	0.0	0.0	4.2				*Elaeagnaceae.* Berries from Montana

The two cactus plants mentioned are not food plants, but are used by the Indians of Texas and Mexico for their intoxicating properties. The nitrogen of these plants is derived from alkaloids present, rather than proteins (2) The silver buffaloberry (*Shepherdia argentea* = *Lepargyrea argentea*) had been analysed by Trimble (12) with results somewhat different from those given here.

TABLE 14.—*Plants of family Apiaceae (Umbelliferae)*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
47	45.1	1.3	15.4	15.2	14.0					*Carum gairdneri** (Hook. and Arn.) A. Gray—Yampa.†
	—	2.4	28.1	27.7	25.5					Roots from California
47a	14.8	4.3	28.0	21.8	5.1	—	—	—	—	Ditto. Wyoming
	—	5.0	32.9	25.6	6.0	—	4.4	—	4.5	
47b	15.8	0.5	29.4	11.7	18.3	—	—	—	—	Ditto. Wyoming
	—	0.6	34.9	13.9	21.7	1.7	6.0	4.0	4.2	
48	7.9	1.5	6.5	5.7	14.2	—	—	—	—	*Cogswellia ambigua*† (Nutt.) Jones. Roots from Utah
	—	1.6	7.1	6.2	15.4	2.3	3.7	37.0	7.5	
49	41.3	0.0	2.0	24.8	18.0	—	—	—	—	*Cogswellia cous* (S. Wats.) Jones. Roots from Washington
	—	0.0	3.4	41.5	30.2	0.3	10.2	7.4	2.0	
49a	33.1	1.2	3.1	12.4	6.2	—	—	—	—	Ditto. Oregon
	—	1.8	4.6	18.5	9.3	—	5.6	—	10.3	
50	93.1	3.0	0.0	0.0	0.8	—	—	—	—	*Heracleum lanatum* Michx.—Common Cow-parsnip
	—	43.5	0.0	0.0	11.6	—	17.7	—	12.6	Leaves and stems from Utah
50a	7.0	4.1	7.8	12.6	5.7	—	—	—	—	Ditto. Stalks and roots from Alaska
	—	4.4	8.4	13.5	6.1	—	9.8	—	11.3	
51	57.8	4.0	3.9	8.8	12.5	—	—	—	—	*Oenanthe sarmentosa* Presl. Tubers from Oregon
	—	9.5	9.2	20.9	29.6	—	5.7	—	5.3	

* Some seeds of *Carum gairdneri* were planted and grown for the writers near Seattle, Wash. The roots were analysed at stated intervals. The results are given in Table 15.

† An old analysis of Yampa can be found in Trimble's series (16). He also analysed roots closely related to the above Cogswellia roots, *Cogswellia canbyi* (Coult. and Rose) Jones (=*Peucedanum canbyi*) (15) and *Cogswellia macrocarpa* (Nutt.) Jones (=*Peucedanum eurycarpum*) (14).

TABLE 15.—*Composition of carum gairdneri roots at different stages of growth*

DATE OF ANALYSIS	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMI-CELLULOSE	PROTEIN	ASH
3/23/31	83.0	0.8	—	4.2			
	—	4.7	—	24.7			
4/ 9/31	80.3	0.0	0.0	4.9			
	—	0.0	0.0	24.9	—	7.7	—
4/23/31	66.1	0.6	1.0				
	—	1.8	2.9				
5/ 6/31	77.0	0.4	1.8	9.6	5.4		
	—	1.7	7.8	41.7	23.5	10.5	7.8
5/21/31	67.2	0.2	3.6	—	—		
	—	0.6	11.0	—	—	9.8	3.5
6/ 8/31	62.3	0.1	3.5	12.8	18.8	—	—
	—	0.3	9.3	34.0	49.9	4.9	2.5
6/22/31	60.3	0.2	3.1	13.7	16.2	—	—
	—	0.5	7.8	34.5	40.8	6.3	3.0
7/13/31	55.4	0.6	5.4	17.5	10.0	—	—
	—	1.3	12.1	39.2	22.4	5.9	2.8
7/29/31	57.5	0.5	12.2	8.9	10.4	—	—
	—	1.2	28.7	20.9	24 5	10.2	3.8
8/20/31	59.2	0.4	11.6	10.6	12.2	—	—
	—	1.0	28.4	26.0	29.9	10.5	4.1
9/ 7/31	75.6	0.3	7.3	8.5	7.7	—	—
	—	1.2	29.9	34.8	31.6	15.1	—
9/28/31	69.0	0.2	7.1	5.9	11.6	—	—
	—	0.6	22.9	19.0	37.4	9.8	—

In Table 12 are given comparative results of analyses of ash of *Glycyrrhiza lepidota* and *G. glabra.*

The roots of balsamorhiza were analyzed after the heavy bark had been taken off. The non-reducing sugar of the plant is inulin (21). The bark of the root contains a sesquiterpene alcohol (19).

Jerusalem artichoke and dandelion are known to be inulin-bearing plants, and have been repeatedly (particularly the former) analyzed by various investigators.

Wyethia roots can be apparently classed as inulin plants, since on hydrolysis of the solution containing the sugars of the plant the initial negative rotation (−1.1°V) changed to a higher negative rotation (−5.0°V).

It may be of interest to note here that about 90 years ago French scientists and the Government officials were very much interested in the roots of *Apios tuberosa* and *Psoralea esculenta* (Table 11). Botanists were sent to this country to collect samples of these plants and there was some talk of replacing the disease-ridden potato crop with either of these plants (3, 4, 5, 7, 9, 11).

Table 16.—*Plants of the families Lennoaceae, Ericaceae, Gentianaceae, Asclepiadaceae, Solanaceae and Cucurbitaceae*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	PROTEIN	ASH	SPECIES AND ORIGIN
52	89.7	2.9	0.0	0.9	6.9	—	—	*Ammobroma sonorae* Torr.—Sandroot. Fam. *Lennoaceae.* Roots from California
	—	28.2	0.0	8.7	61.2	—	4.8	
53	80.6	0.3	0.6	3.4	8.4	—	—	*Pholisma arenarium* Nutt. Fam. *Lennoaceae.* Roots from California
	—	1.5	3.1	17.5	43.3	3.1	5.3	
54	12.8	18.6	0.0	0.0	13.1			*Arctostaphylos tomentosa* (Pursh) Lindl.—Woolly Mansanita. Fam. *Ericaceae.* Fruit from California
	—	21.3	0.0	0.0	15.0			
55	58.0	7.9	1.3	0.0	8.4			*Arctostaphylos uva-ursi* (L.) Spreng.—Bearberry. Fam. *Ericaceae.* Fruit from Utah
	—	18.8	3.1	0.0	20.0			
55a	15.7	22.0	1.6	0.0	9.4			Ditto. Colorado
	—	26.1	1.9	0.0	11.2			
56 (roots)	6.3	1.6	0.7	0.0	11.7	—	—	*Frasera speciosa* Dougl. Fam. *Gentianaceae.* Roots and tops from Texas
	—	1.7	0.7	0.0	12.5	3.5	6.4	
56 (tops)	8.6	2.0	0.7	0.0	9.6	—	—	
	—	2.2	0.8	0.0	10.5	8.4	12.3	
57	8.1	5.9	6.0	4.3	9.5	—	—	*Asclepias tuberosa* L.—Butterflyweed. Fam. *Asclepiadaceae.* Roots from Mississippi
	—	6.4	6.5	4.7	10.3	5.3	13.9	
57a	5.9	5.6	5.3	0.0	12.3	—	—	Ditto. Tops from Alabama
	—	6.0	5.6	0.0	13.1	10.2	7.5	
57b	13.1	9.1	9.5	11.5	7.8	—	—	Ditto. Roots from Illinois
	—	10.5	10.9	13.2	9.0	7.2	9.0	
57c	6.9	1.3	1.4	19.2	12.4	—	—	Ditto. Roots from Alabama
	—	1.4	1.5	20.6	13.3	8.3	8.6	
58	78.3	1.4	4.6	0.0	2.6	—		*Physalis longifolia* Nutt. Fam. *Solanaceae.* Fruit from Nebraska
	—	6.4	21.2	0.0	12.0	15.0		
59 (fruit)	89.9	1.5	0.0	0.0	2.4	—	—	*Cucurbita foetidissima* H.B.K.—Buffalo Gourd. Fam. *Cucurbitaceae* Fruit and roots from Texas
	—	14.9	0.0	0.0	23.8	23.1	9.5	
59 (roots)	65.3	2.2	0.0	9.0	6.6	—	—	
	—	6.3	0.0	25.9	19.0	20.4	4.9	

TABLE 17.—*Plants of family Asteraceae (Compositae)*

PLANT	MOISTURE	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	HEMICELLULOSE	ETHER EXTRACT	PROTEIN	CRUDE FIBER	ASH	SPECIES AND ORIGIN
60	7.8	5.4	8.0	0.0	14.0					*Balsamorhiza deltoidea* Nutt. Roots from California
	—	5.8	8.7	0.0	15.2					
61	7.0	10.6	9.3	0.0	10.8	—	—	—	—	*Balsamorhiza sagittata* (Pursh) Nutt.—Arrowleaf Balsamroot. Roots from Utah
	—	11.4	10.1	0.0	11.6	5.8	3.5	49.2	6.0	
61a	9.0	12.4	9.0	0.0	10.0					Ditto. Wyoming
	—	13.6	9.9	0.0	11.0					
61b	8.8	2.1	6.2	0.0	13.5	—	—	—	—	Ditto. Utah
	—	2.3	6.8	0.0	14 8	3.6	2.1	—	6.6	
61c	7.6	7.3	11.2	0.0	14.6	—	—	—	—	Ditto. Nevada
	—	7.9	12.1	0.0	15.8	—	5.1	—	5.0	
61d	7.2	5.4	7.5	0.0	11.6	—	—	—	—	Ditto. Wyoming
	—	5.8	8.1	0.0	12.5	—	5.9	—	6.3	
62	70.8	0.3	0.3	0.0	4.4					*Cirsium drummondii* Torr. and Gray. Roots from Colorado
	—	1.0	1.0	0.0	15.1					
62a	85.2	trace	trace	0.0	2.2	—	—	—	—	Ditto. Utah
	—	trace	trace	0.0	14.9	—	12.6	—	7.5	
63	70.2	0.0	20.5	0.0	2.3	—	—	—	—	*Helianthus tuberosus* L.—Jerusalem-artichoke. Tubers grown in District of Columbia
	—	0.0	68.8	0.0	7.7	—	12.5	—	3.3	
64	48.8	1.0	12.8	0.0	7.2					*Leontodon taraxacum* L.—Dandelion. Roots from District of Columbia
	—	2.0	25.0	0.0	14.1					
65	27.7	4.2	16.1	—	—	—	—	—	—	*Wyethia amplexicaulis* Nutt. Roots from Oregon
	—	5.8	22.3	—	—	—	9.0	—	6.0	
66	58.0	2.7	12.3	0.0	4.7	—	—	—	—	*Wyethia sp.* Roots from Colorado
	—	6.4	29 3	0.0	11.2	—	4.2	—	6.8	

SUMMARY AND CONCLUSIONS

Analyses of 119 Indian food plants of 66 species are presented in this paper. These analyses are not extensive enough to enable one to draw definite conclusions as to whether there is any vital difference between present-day diet and that of the aborigines. Some interesting facts, however, may be pointed out here. Out of 66 species analyzed, six were found to contain inulin as a carbohydrate reserve material. Among these six plants was camas root—one of the most popular foods of the northwestern Indians. Inulin is practically absent in foods used in this country at the present time.

Another peculiar constituent of Indian diet was lichenin of the lichens (*Alectoria jubata*). Attention is called to the abnormally high calcium content of hackberries (*Celtis occidentalis*). It may also be mentioned that some foods, e.g., *Aesculus californica*—California buckeye, which are considered poisonous or obnoxious by the white people were by proper cooking made into very palatable foods by the Indians.

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DETERMINATION OF CHLORATE IN SOIL EXTRACTS, CULTURE SOLUTIONS, AND PLANT SAP[1]

By RICHARD S. ROSENFELS[2] (California Agricultural Experiment Station, Davis, Calif.)

The methods described in this paper were developed during work at this Station on the use of sodium chlorate as an herbicide. The paper is in two parts: (a) the adaptation of the sulfurous acid reduction method to the analysis of extracts of soils containing sodium chlorate, and (b) the adaptation of Ferrey's iodometric method (6) to the analysis of culture solutions and xylem sap of squash.

THE SULFUROUS ACID REDUCTION METHOD FOR DETERMINATION OF CHLORATE

Investigators in the field of weed control have experienced difficulty in finding methods for the determination of chlorate suited to the analysis of soil extracts. The method of reduction with a measured excess of ferrous sulfate followed by titration of the excess with permanganate has been used, but the permanganate titration is apt to be too high due to reducing

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[1] This paper was made possible by the cooperative project on control of noxious weeds conducted by the California Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

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substances naturally present in the extract. Åslander (1) employed this method, but does not report recovery tests on known quantities of chlorate. Bowser and Newton (2) found by such tests that low results were obtained due to the above-mentioned effect. Loomis, Smith, Bissey, and Arnold (7) found the iodometric method unsatisfactory. They tested for chlorate by growing plants and observing the extent of injury. Biological tests for chlorate have been used by others. Since such tests indicate toxicity, which may be the resultant of several chemical factors, they cannot in the final analysis be a satisfactory substitute for specific chemical determination.

The sulfurous acid reduction method here described is a modification of the method of Ensink and Hofman (5). It is based on the reduction of chlorate to chloride by sulfurous acid. The chloride is determined before and after reduction by the Mohr titration with silver nitrate, potassium chromate being used as indicator. The difference in titer before and after reduction is a measure of the amount of chlorate, each mol of silver nitrate representing a mol of chlorate.

The method is as follows:

Pipet a 10 ml. portion of a 1:1 extract of the soil into a 250 ml. Erlenmeyer flask. Add 5 ml. of C.P. sulfurous acid reagent (6% SO_2) followed by about 35 ml. of water. Boil the mixture until the volume is reduced to 15–20 ml. Again add water and boil down, and if necessary repeat the boiling once more.[1] Cool the solution and add 1 drop of 0.04% aqueous methyl orange indicator solution. Add 0.1 N NaOH until the first distinct yellow appears. Add water to make a total volume of 50 ml. Add 1 ml. of 0.3 M K_2CrO_4, and titrate with 0.03 N $AgNO_3$.

To another 10 ml. aliquot add 0.1 N H_2SO_4 equal to the volume of 0.1 N NaOH used in the reduced sample. Dilute with 20–30 ml. of water. Add the methyl orange indicator just before neutralizing with 0.1 N NaOH. (The indicator may be oxidized by the chlorate in acid solution if allowed to stand too long before the NaOH is added.) Add water to make 50 ml. as before. Add chromate indicator and titrate to determine the original chloride content.

To standardize the $AgNO_3$ solution take 5 ml. of 0.03 N NaCl; add 40 ml. of water, 1 drop of methyl orange indicator, and 1 ml. of 0.3 M K_2CrO_4; and titrate. Subtract a blank run on 50 ml. of water containing 1 drop of the methyl orange solution.

Sulfuric acid, subsequently neutralized with sodium hydroxide, is added to the non-reduced sample because a positive titration error may result from the sodium sulfate present in the reduced sample. This error is apparently due to the effect of the salt upon the physical state of the silver chloride precipitate, and not to chloride impurity. The magnitude of the error depends roughly on the amount of sulfuric acid present after reduction. This in turn depends on the amount of sulfuric acid present as an impurity in the sulfurous acid solution, and on the amount of chlorate, one mol of chlorate producing theoretically six equivalents of

[1] The solution need not be boiled longer than necessary to remove the excess SO_2. In the tests here reported the solutions were boiled down a total of three times to provide a margin of safety.

hydrogen ion in the reduction. The sulfuric acid resulting from chlorate reduction will vary from sample to sample, and that present as impurity in the reducing agent will increase with time, due to spontaneous oxidation. The simplest way to allow for these variations, apparently, is to add sulfuric acid to the non-reduced sample as indicated.

Even with this precaution a titration error may not be obtained on the non-reduced sample if the soil extract contains little or no chloride.

TABLE 1.—*Recovery of sodium chlorate by the reduction method in the presence of 10 ml. of 1:1 extract of Fresno sandy loam*

TREATMENT	ClO_3^- TAKEN	0.1 *N* NaOH REQUIRED	0.03 *N* $AgNO_3$ TITRATION	ClO_3^- RECOVERED		TITRATION ERROR
	millimols	*ml.*	*ml.*	*millimols*	*per cent*	*ml.*
Reduced	0.020	2.84 2.83	0.85 0.85	0.022	110	+0.09
	0.050	4.83 4.85	1.90 1.88	0.054	108	+0.12
	0 100	8.58 8.11	3.60 3.58	0.105	105	+0.16
	0.200	14.58 14.68	6.95 6.95	0.205	102	+0.18
	None	1.38 1.28	0.10 0.10			
Not reduced	0.200*	2.72 2.70	0.10 0.10			
	0.200†	14.31 14.33	0.10 0.10			

* 2.85 ml. 0.1 *N* H_2SO_4 added
† 14.65 ml. 0.1 *N* H_2SO_4 added.

This is because (a) there is no masking of the red silver chromate color in the absence of an appreciable silver chloride precipitate, and (b) the sodium sulfate is without effect under these circumstances. High results will be obtained when there is no titration error on the non-reduced sample corresponding to the error on the reduced sample. This point is illustrated in Tables 1 and 2.

The method as outlined is suited to the determination of amounts of chlorate as small as 0.02 millimols (2.1 milligrams sodium chlorate). Much larger amounts can also be determined, a 10 ml. titration difference between reduced and non-reduced samples corresponding to 0.3 millimols.

If desired, a more concentrated silver nitrate solution could be used for the determination of still larger amounts of chlorate.

Table 1 gives the results of recovery tests by the reduction method with 10 ml. of a 1:1 water extract of Fresno sandy loam present in each flask. Each determination was made in duplicate.

It was unnecessary, in this series, to run non-reduced samples for each amount of chlorate because the minimum silver nitrate titration possible was obtained on the non-reduced sample containing the largest quantity of chlorate (0.200 millimols) and the largest addition of 0.1 *N* sulfuric acid (14.65 ml.). This minimum titration of 0.10 ml., obtained on the

TABLE 2.—*Data of Table 1 recalculated to show recovery of sodium chlorate based on differences between amounts originally taken*

DIFFERENCE BETWEEN AMOUNTS OF ClO_3^- TAKEN	DIFFERENCE BETWEEN $AgNO_3$ TITRATIONS	ClO_3^- RECOVERED	
millimols	*ml.*	*millimols*	*per cent*
0.03 (.05 − .02)	1.04	0.031	103
0.05 (.10 − .05)	1.70	0.051	102
0.10 (.20 − .10)	3.36	0.101	101
0.18 (.20 − .02)	6.10	0.183	102

last six samples of Table 1, is equivalent to the blank titration on pure water. The extract of this particular soil is thus free of chloride, and the non-reduced silver nitrate titrations are identical, regardless of the fact that one pair was performed in the presence of much more sodium sulfate than the other. This illustrates the fact, referred to above, that a titration error is not produced by sodium sulfate in the absence of chloride. The high recoveries obtained, especially with 0.020 and 0.050 millimols of chlorate, show that a titration error did occur in the presence of chloride. The titration errors are recorded in the last column of Table 1, the smallest causing the largest percentage error in recovery.

The errors in recovery would apparently be lower if chloride were present in the non-reduced samples. Experimental addition of chloride was not tried, but the same effect was obtained by determining the differences in silver nitrate titrations corresponding to differences in amounts of chlorate taken. Table 2 gives the results of these calculations. Each titration was performed in the presence of chloride, and the net errors are appreciably lower than in Table 1.

These results indicate that in the analysis of chloride-free extracts containing small amounts of chlorate, it is advisable to introduce a small

amount of chloride into both reduced and non-reduced samples. The difference in silver nitrate titration between reduced and non-reduced samples will then indicate the amount of chlorate as before, and both titrations will be subject to an error.

TABLE 3.—*Recovery of sodium chlorate by the reduction method in the presence of 10 ml. of 1:1 extract of Yolo clay loam*

ClO_3^- TAKEN	TREATMENT	0.03 *N* $AgNO_3$ TITRATION	ClO_3^- RECOVERED	
millimols		*ml.*	*millimols*	*per cent*
0.020	Reduced	0.95 0.90	0.020	100
	Not reduced	0.25 0.25		
0.050	Reduced	1.92 1.93	0.050	100
	Not reduced	0.25 0.25		
0.100	Reduced	3.65 3.67	0.102	102
	Not reduced	0.25 0.25		
0.200	Reduced	7.00 7.02	0.202	101
	Not reduced	0.28 0.26		
None	Reduced	0.22 0.22		
	Not reduced	0.25 0.22		

The determination of chlorate by measurement of the amount of sulfuric acid produced in the reduction was considered, but the data of Table 1 indicate that this method is not reliable. Three of the duplicate titrations with 0.1 *N* sodium hydroxide differ by 0.1 ml. or more. Furthermore, the amount of sulfuric acid produced is in every case greater than theoretical for the amount of chlorate taken. Tests on pure chlorate solutions, not included in this paper, gave similar results.

Table 3 gives the results of recovery tests by the reduction method on 10 ml. of a 1:1 extract of Yolo clay loam, a soil containing an appreciable,

though small, quantity of chloride. In this series the directions for the method were followed in detail as previously given, a pair of non-reduced samples being run with each pair of reduced samples. Each non-reduced sample was given the volume of 0.1 N sulfuric acid required by the corresponding reduced sample, and this was neutralized with 0.1 N sodium hydroxide.

The improved results obtained with this soil, as compared with Fresno sandy loam, indicate that the titration errors are equal, or nearly so, on reduced and non-reduced samples. Good results were also obtained on a third soil of about the same chloride content as Yolo clay loam. Apparently a small amount of chloride is sufficient to give satisfactory results.

The silver nitrate titrations on the non-reduced samples in Table 3 indicate that increases in the amount of sodium sulfate have but little effect. This is because the amount of chloride was small. With a total titration of the order of 5 ml., increases in sodium sulfate increase markedly the titration error. The method as outlined is designed to give reliable results on all soils including those of high chloride content.

The apparent difference in titration between the non-reduced samples and those containing no chlorate is probably without significance, especially in view of the difficulty of determining accurately the chromate end-point with a dilute silver nitrate solution.

THE IODOMETRIC METHOD FOR DETERMINATION OF CHLORATE

This method, although more limited in application than the reduction method, is accurate and more rapid. The procedure here described is a modification of the method of Ferrey (6), and is based upon the reduction of chlorate by hydriodic acid followed by titration of the resulting iodine with thiosulfate. The reduction, which will not otherwise proceed, is made possible by the addition of ferrous sulfate. Since iodine is produced in the absence of chlorate under the conditions employed, a blank determination is made and the amount of chlorate determined by difference, each mol of thiosulfate corresponding to 1/6 mol of chlorate.

The method is as follows:

REAGENTS

Potassium iodide.—25% aqueous solution (25 grams per 100 ml. of solution).

Acid ferrous sulfate reagent.—This is 0.315 N with respect to ferrous sulfate, and 6.3 N with respect to sulfuric acid. To prepare dissolve 87.6 grams of $FeSO_4 \cdot 7H_2O$ in water, add 350 ml. 1:1 (18 N) H_2SO_4, and make to 1 liter.

PROCEDURE

Pipet a 10 ml. portion of the chlorate-containing solution into a 250 ml. glass-stoppered bottle. Add 10 ml. of the KI solution followed by 15 ml. of the acid ferrous sulfate reagent. Stopper the bottle and let stand 30 minutes. Add 70 ml. of water, and titrate with 0.03 N thiosulfate to a starch end point. Repeat with 10 ml. of water in place of the chlorate solution. The difference between the titrations is a measure of the amount of chlorate.

In making a run of several determinations have the time interval identical for each. A run of 14 determinations including 2 blanks and 12 unknowns may be conveniently made as follows: Introduce the KI and experimental solutions into each bottle, and use Nos. 1 and 14 as blanks. Add the acid ferrous sulfate to each bottle in order at intervals of 2 minutes. Start the titration on bottle No. 1, 4 minutes after the last addition of acid ferrous sulfate, and continue the titrations in order at intervals of 2 minutes. (The blank titrations should agree within the limits of error of the determination. If the titrations exceed 4 or 5 ml. it may be necessary to allow more than 2 minutes to complete them, and the foregoing schedule can be altered accordingly.)

The thiosulfate solution can be conveniently standardized with 0.03 N (0.005 M) $KBrO_3$. Combine 5 ml. of the $KBrO_3$ solution, 10 ml. of 25% KI, 5 ml. of 4 N HCl, 10 ml. of water, and 3 drops of 3% sodium molybdate solution. Let stand a minute or two, add 50 ml. of water, and titrate

TABLE 4.—*Age of the acid ferrous sulfate reagent in relation to the magnitude of the blank titration in the iodometric method of chlorate determination (determinations in duplicate)*

AGE OF ACID FERROUS SULFATE REAGENT	BLANK TITRATION 0.03 N $Na_2S_2O_3$
	ml.
9 months	52.90
	52.90
1 month	7.40
	7.45
2 hours	0.60
	0.60

Chlorate in amounts of from 0.005 to 0.05 millimols can be satisfactorily determined by the procedure outlined, and much larger amounts could be determined by increasing the strength of the thiosulfate solution.

As indicated by Ferrey (6), light is an important factor in the oxidation of hydriodic acid. The blanks and unknowns should therefore be subjected to approximately the same light intensity. In the tests here reported, the bottles were allowed to stand in diffused daylight, the direct rays of the sun being avoided.

Difficulty has been experienced by some investigators in controlling the blank titration (see Loomis, et al (7), for example). The tests reported in Table 4 indicate that the blank titration increases with increasing age of the acid ferrous sulfate reagent. This is probably due to the oxidation of ferrous to ferric ions as the solution stands. A blank titration in excess of 1 to 2 ml. is objectionable, and can be easily eliminated by substituting a freshly prepared acid ferrous sulfate reagent.

Table 5 gives the results of recovery tests by the iodometric method in the presence of 10 ml. of culture solution[1] and 10 ml. of 1:1 extract of

[1] This solution had the following composition: $Ca(NO_3)_2$, 0.004 M; KNO_3, 0 006 M; $MgSO_4$, 0 002 M; and $(NH_4)H_2PO_4$, 0.001 M. Smaller amounts of Fe, Mn, B, Zn, and Cu.

the same soils used in the tests on the reduction method. The determinations were made in duplicate and are listed in the order performed. Five ml. of 50 per cent potassium iodide was used, and the chlorate was introduced as 5 ml. of 0.002 *M* sodium chlorate. Two blanks were run on water, and one each on the culture solution and two soil extracts. The latter contained 10 ml. of the solution or extract concerned, but no chlorate.

TABLE 5.—*Recovery of 0.010 millimols of sodium chlorate by the iodometric method in the presence of 10 ml. of culture solution*[1] *and 10 ml. of 1:1 extract of Fresno sandy loam and Yolo clay loam*

SAMPLE	0.029 *N* $Na_2S_2O_3$ TITRATION	ClO_3^- RECOVERED	
	ml.	*millimols*	*per cent*
Blank—water	0.95		
Blank—culture solution	0.95		
Chlorate plus culture solution	3.05 3.05	0.0101	101
Blank—extract Fsl	1.05		
Chlorate plus extract Fsl	3.15 3.15	0.0101	101
Blank—extract Ycl	1.85		
Chlorate plus extract YCl	3.95 3.85	0.0099	99
Blank—water	1.00		

Table 5 indicates that satisfactory recoveries can be obtained from soil extracts. However, the blank titration on the extract of Yolo clay loam was nearly twice as great as that on water, indicating the presence of substances capable of oxidizing hydriodic acid under the conditions employed. The analysis of a field sample of soil containing chlorate would thus necessitate the preparation of an extract of an otherwise identical sample containing no chlorate. The obvious difficulties of such a requirement render the iodometric method unsuitable for use on soils which behave like Yolo clay loam. The blank titration on Fresno sandy loam was but slightly higher than that on water, and the possibility exists that this method could be employed on certain soils. However, the reduction method already described eliminates the possibility of difficulty from oxidizing substances, and although more time-consuming, appears better adapted in general to soil-extract analysis.

The results on the culture solution given in Table 5 indicate that the iodometric method operates satisfactorily in the presence of amounts of nitrate equal to several times the amount of chlorate, the blank titrations being identical on water and culture solution.

Further recovery tests on culture solution are given in Table 6. The determinations were again made in duplicate, and are listed in the order performed. Five ml. of 50 per cent potassium iodide was used, and each of the varying amounts of chlorate was introduced in a 5 ml. aliquot. Four blanks, each on culture solution, were included. The results recorded are typical of others obtained in unreported tests on chlorate recovery from water.

TABLE 6.—*Recovery of sodium chlorate by the iodometric method in the presence of 10 ml. of culture solution*[1]

ClO_3^- TAKEN	0.029 *N* $Na_2S_2O_3$ TITRATION	ClO_3^- RECOVERED	
millimols	*ml.*	*millimols*	*per cent*
Blank	0.85		
0.005	1.85 1.87	0.0049	98
Blank	0.85		
0.025	6.00 5.98	0 0248	99
Blank	0.87		
0.050	11.07 11.07	0 0494	99
Blank	0.85		

The iodometric method is well suited to the analysis of culture solutions, and might be advantageously substituted for the longer procedures that have been used on this type of solution by Cook (3), for example, and others.

The iodometric method, as here described, has been used by the writer for the analysis of xylem sap of squash plants that had absorbed sodium chlorate from culture solution (4). Recovery tests with known amounts of chlorate were not made, but it was repeatedly observed that the blank titration was the same on water as on chlorate-free xylem sap provided the sap samples were fresh. If allowed to stand a few days, whether refrigerated or not, the sap samples undergo changes resulting in increased blank titrations and indefinite titration end points.

The method is apparently well suited to the analysis of fresh samples of this particular plant sap, and might be used on similar biological materials such as the *Nitella* sap analyzed by Offord and d'Urbal (8). If it proved applicable, the iodometric method would be faster and more convenient than the procedure they employed. Its suitability may be determined in any particular instance by comparing blank titrations on water and on the material concerned. If these are equal the method can, in all likelihood, be used.

SUMMARY

Two methods of chlorate determination are described. One, called the reduction method, is based upon the reduction of chlorate to chloride by sulfurous acid. The chloride is determined before and after reduction by titration with silver nitrate, the difference being a measure of the amount of chlorate. Recovery tests on known amounts of chlorate show that the method gives reliable results on soil extracts.

A shorter procedure, called the iodometric method, is based upon the reduction of chlorate by hydriodic acid followed by titration of the resulting iodine with thiosulfate. Recovery tests show that the method is not suited to the analysis of soil extracts, but gives reliable results on culture solution. It is also satisfactory for the analysis of xylem sap of squash.

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OBSERVATIONS ON THE DETERMINATION OF ASH IN FEEDING STUFFS*

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In the routine testing of mixed feeds for ash or mineral content, the methods in use are those of the Association of Official Agricultural Chemists or the American Association of Cereal Chemists. The A.O.A.C. method specifies ignition of the sample at a dull red heat until it is carbon free. The A.A.C.C. requires incineration at 550° C. until the sample is light gray in color or no further loss in weight occurs.

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During the past year, in the work of feed collaborative testing, the writer noticed that when the samples contained calcium carbonate, the results among laboratories were extremely irregular. Comments by collaborators were "hard to check," and "ash seems to evaporate."

A survey of methods for ash determination was therefore conducted among the collaborating members of the feed committee, and it was found that there was a marked divergence of opinion in regard to the point at which the sample ceases to lose weight, as measured by the time of ignition, when calcium carbonate is present, and also as to what constitutes "dull red heat."

In an effort to solve some of these questions, a series of ash determinations was made on the grains and by-products commonly used in mixed feeds to determine the effect of time and temperature.

Corn, wheat, oats, meat meal, fish meal, and millrun were selected as representing the whole grains and by-products utilized by the feed mixer. Duplicate 2.0 gram samples were ashed for 15–18 hours at 550° and 650° C., and for 2 hours at 700° C. The results are shown in Table 1.

TABLE 1.—*Results of ashing at varying temperatures and for varying periods*

	550° C. 15–18 HRS.	650° C 15–18 HRS.	700° C. 2 HRS.
Wheat	1.58	1.59	1.58
Corn	1.91	1.95	1.95
Oats	3.05	3.06	3.09
Meat meal	26.87	28.75	28.04
Fish meal	10.19	10.12	10.02
Millrun	5.38	5.69	5.26

These results (Table 1) show that satisfactory checks can be obtained by either the A.A.C.C. or the A.O.A.C. method (650° C. being slightly higher than dull red). For a rapid test, 700° C. for 2 hours yields comparable results with the official method on commodities such as those tested.

Although the amount of calcium carbonate used in a mixed feed is ordinarily small, it may constitute 15–30 per cent of the total ash. Therefore, any lack of uniformity in the decomposition of this compound will cause variable ash results.

As a means of determining the decomposition of calcium carbonate under varying conditions, duplicate 2.0 gram samples of oyster-shell flour, limestone flour, and C. P. calcium carbonate were ashed in the same manner as were the grains and by-products listed in Table 1. The results are shown in Table 2.

In this series of determinations (Table 2) the duplicates were "spotted" irregularly over the floor of the oven. It will be noted that at 550° C. overnight and at 700° C. for 2 hours, the decomposition was very irregular, depending on the location of the sample in the oven and that there-

fore it was impossible to obtain concordant results. At 650° C. overnight, the time and temperature were such that practically complete decomposition of the calcium carbonate took place.

As a further check, seven samples of C. P. calcium carbonate were ashed simultaneously at 700° C. for 2 hours. Four samples in the center of the oven yielded the theoretical amount of calcium oxide. Samples in the back and the front of the oven were significantly higher in total ash; that is, but a partial conversion to calcium oxide took place.

TABLE 2.—*Decomposition of calcium carbonate under varying conditions*

	550° C. 15–18 HRS.	650° C. 15–18 HRS.	700° C. 2 HRS.
	per cent	*per cent*	*per cent*
Limestone flour*	96.45		63.90
	87.85	57.53	58.13
	92.15		61.01
Shell flour*	80.65		57.24
	97.68	56.90	73.95
	89.16		65.59
Tailings*	78.11	55.72	56.03
C. P. calcium carbonate	98.62	56.20	88.30
	92.00		58.23
	95.31		73.26

* Through 28 wire.

From these tests and a knowledge of the ashing methods in use, it was concluded that variations in ash results among laboratories on samples containing calcium carbonate were due to non-uniform decomposition of the lime compounds, which in itself is caused by time and temperature differences in these laboratories.

To substantiate this conclusion, the writer sent to the members of the feed collaborative committee two samples, one containing 90 per cent whole wheat flour and 10 per cent C. P. calcium carbonate, the other 80 per cent whole wheat flour and 20 per cent calcium carbonate, with instructions to ash 2.0 gram samples 15–18 hours at 550° and 650° C., and also 2 hours at 700° C. The results are shown in Table 3. A mixed feed sample was submitted at the same time with no instructions. This sample was also used by the writer and Broom for determining ash at different temperatures.

The results are outlined in Table 4.

TABLE 3.—*Results (per cent) on whole wheat flour and calcium carbonate mixtures*

METHOD	CLULOW	TUTTLE	MACDONALD	KING	DE HAAN	JOUKOVSKY	BROOM	HUBER
Sample A—90% Whole Wheat flour+10% C. P. calcium carbonate								
2 g. 550° C. 15–18 hrs.	10.77	11.10	7.57	7.03	625° C. 7.18		7.11	9.02
2 g. 650° C. 15–18 hrs.	6.95	7.20	13.39	6.95	7.13	no tempera-ture control	7.17	8.15
2 g. 650° C. 2 hrs.	7.12							
2 g. 700° C. 2 hrs.	7.02	7.40	8.24	6.97	7.16	7.02	6.94	7.13
2 g. 730° C. 16 hrs.							7.14	
Sample B—80% whole wheat flour+20% C. P. calcium carbonate								
2 g. 550° C. 15–18 hrs.	20.00	20.70	13.43	12.45			12 80	17.90
2 g. 625° C. 15–18 hrs.					12.53			
2 g. 650° C. 15–18 hrs.	12.33	14.50	20.69	12.35	12.41	no tempera-ture control	12.49	19.50
2 g. 650° C. 2 hrs.	12.58							
2 gr. 700° C. 2 hrs.	12.34	14.65	14.08	17.51	12.54	12.31	12.38	12.65
2 g. 730° C. 16 hrs.							12.42	

Calculated ash:	*Sample A* *per cent*	*Sample B* *per cent*
No decomposition	11.52	21.35
100% decomposition	7.12	12.55

On the mixed feed sample, with one exception, the collaborating laboratories checked within the experimental error. The tests on the whole wheat flour and calcium carbonate mixture brought out the fact that at a temperature of 550° C. the oven control was very erratic as results ranged from 17 to 100 per cent decomposition of the calcium carbonate. At higher temperatures the results are somewhat more uniform, but there is still considerable room for improvement.

TABLE 4.—*Results on mixed feed sample*

COLLABORATORS		GLULOW	BROOM	
	per cent	*per cent*	*per cent*	
Fluke	6.23	6.51	6.46	550°—16 hrs.
MacDonald	7.50			
Linnett	6.15	5.49	5.72	650°—16 hrs.
Huber	6.50			
DeHaan	6.30	6.23		650°— 2 hrs.
Tuttle	6.40			
Joukovsky	6.45	5.85	5.45	700°— 2 hrs.
King	6.20			
		Range	1.35	
		Average	6.32	

It is apparent, therefore, that with the equipment available in the average laboratory more satisfactory analyses for ash will be obtained by utilizing a temperature sufficiently high to completely decompose the calcium carbonate into calcium oxide.

That this procedure is justifiable from the standpoint of state inspection is evident from correspondence with L. S. Walker, Referee on Feeding Stuffs for the A.O.A.C., who states that "A dull red heat would naturally make the finished ash contain calcium oxide."

A CHEMICAL EXAMINATION OF THE LIGNIN-LIKE SUBSTANCE FROM THE SPOROPHORES OF *FOMES PINI* (THORE) LLOYD (*TRAMETES PINI* (THORE) FR.)

By MAX PHILLIPS*

Fomes pini (Thore),[1] also known as *Trametes pini* (Thore) Fr., is a bracket-shaped fungus that is found rather widely distributed throughout the world. This fungus attacks nearly all coniferous trees and produces what is known as white pocket rots. It is stated by Hartig,[2] that *F. pini* preferentially attacks the lignin of wood, leaving white patches of unattacked or partially attacked cellulose. Hubert,[3] found that the action of *F. pini* on wood is chemical or enzymatic in character, and that the enzyme first attacks and dissolves the lignin complexes, leaving for the most part cellulose-like fibers.

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* Contribution No. 287, Industrial Farm Products Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

[1] Lloyd, Mycological Writings, Synopsis of the *Genus Fomes*, vol. 4, p. 274, Cincinnati, Ohio (1915); Overholts, Wash. Univ. (St. Louis, Mo.) Studies III, part I, No. 1 (1915); Shope, *Annals Missouri Botan. Garden* (St. Louis, Mo.), 18, 287 (1931); Wolf, Univ. Iowa Studies in Natural History, 14, No. 1 (1931).

[2] Hartig (translation by Wm. Somerville), Text-Book of the Diseases of Trees, p. 193, London (1894).

[3] Hubert, An Outline of Forest Pathology, pp. 406–7. Wiley, New York (1931).

Spaulding[1] examined wood infected with *F. pini* and concluded that the principal action was a delignifying type, and that the white fibers present in the later states of decay were composed mainly of cellulose.

Johnsen and Lee[2] reported that wood decayed by *F. pini* showed a loss of 30 per cent of its lignin and a corresponding increase in its percentage of cellulose.

In connection with a preliminary investigation of the lignin-like complexes in certain fungi, made by Thom and Phillips,[3] it was found that when a sporophore of *F. pini* was treated with fuming hydrochloric acid there was obtained an insoluble lignin-like residue that amounted to 54.0 per cent of the starting material. This product, unlike lignin, contained practically no methoxyl. No further characterization was possible at that time, because of the limited quantity of material at hand. Later when several large specimens of *F. pini* were available,[4] the writer made a more extended study of this material, the results of which are presented in this paper.

The lignin-like[5] material was isolated from the sporophores of *F. pini* by the fuming hydrochloric acid method, and some of its chemical properties were determined. In corroboration of the previous findings this material contained practically no methoxyl groups. It also contained a somewhat smaller percentage of both carbon and hydrogen than does lignin.[6] However, this lignin-like material does undergo a number of reactions that are qualitatively quite similar or analagous to those given by lignin. Thus, on chlorination, a derivative containing approximately 36 per cent of chlorine was obtained. Powell and Whittaker[7] chlorinated alkali lignin and obtained a product with 35.1 per cent of chlorine. On bromination, a derivative was obtained, which, when boiled with 5 per cent sodium acetate solution, lost 71.5 per cent of its bromine. Fuchs[8] obtained similar results with Willstätter lignin. Acetylation afforded a derivative with 24.8 per cent of acetyl, which is somewhat higher than the percentage generally found in acetylated lignin.[9] However, in view of the unmethylated character and presumably greater percentage of free hydroxyls, this is what might be expected. Methylation with diazomethane gave a product that contained 16 per cent of methoxyl. This result corresponds closely to the percentage of methoxyl found in lignin isolated from certain woods.[10] It is well known that the methoxyl content of

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[1] Ann. Rept. Missouri Botan. Garden (St. Louis, Mo.), 17, 41 (1906).
[2] *Pulp Paper Mag. Can.*, 21, 111 (1923).
J. Wash. Acad. Sci. (Washington, D. C.), 22, 237 (1932).
[4] The writer expresses his thanks to Drs. Carl Hartley and G. G Hedgcock of the Division of Forest Pathology, Bureau of Plant Industry, U S. Department of Agriculture, who kindly furnished the *F pini* sporophores used in this investigation.
[5] The term "lignin-like" is used in this paper to designate the product obtained from *F pini* by the fuming hydrochloric acid method used for the isolation of lignin from the seed plants It is so called because of its similarity to lignin in composition and chemical properties.
[6] Phillips, *Chem. Rev.*, 14, 125 (1934).
[7] *J. Chem. Soc.*, 125, 357 (1924).
[8] *Brennstoff-Chem.*, 9, 348 (1928).
[9] Phillips, *Chem. Rev.*, 14, 129 (1934).
[10] ——, *Ibid.*, p. 126.

lignin varies somewhat with the source and the method used for its isolation. When the material was repeatedly methylated with dimethyl sulfate and alkali, a product was obtained which contained approximately 27 per cent of methoxyl. Heuser and coworkers[1] obtained a similar result with Willstätter lignin isolated from wood. Nitration afforded a product similar to that obtained from lignin. When subjected to alkali fusion, protocatechuic acid (isolated as its dimethyl derivative, veratric acid) as well as acetic acid was obtained.

When ground and extracted sporophores of *F. pini* were heated with methyl cellosolve (monomethyl ether of ethylene glycol) in the presence of hydrochloric acid, a product was obtained, the composition of which was similar to that of lignin isolated from the seed plants by this method.[2]

According to Huber[3] and Longyear[4] fungi producing white rots bring about the delignification of the wood by an enzymatic process, whereby the union between the lignin and the carbohydrates of the wood is split, and the solution of the lignin is then effected by the enzyme ligninase. The exact nature of the mechanism involved in the degradation of the lignin and its conversion into soluble form is not clear, but the results of this investigation show that demethoxylation is certainly one of the steps involved in this process. Oxidation undoubtedly also takes place, and Bavendamm[5] has shown that white-rot fungi invariably produce the enzyme oxidase, whereas brown-rot fungi do not. In view of the results presented in this paper, it would appear that the fungus *F. pini* first resolves the lignin into simpler components and then resynthesizes them into a complex product whose chemical properties are similar to those of lignin.

EXPERIMENTAL

Extraction of the Lignin-like Substance from Fomes Pini.—The sporophores were freed from bark and other extraneous materials, ground in a mill, and exhaustively extracted with a 1:2 alcohol-benzene solution. Fifty grams of the extracted material was mixed with 3 liters of 1 per cent hydrochloric acid and boiled under a reflux condenser for 4 hours. The mixture was filtered, washed with water until free of hydrochloric acid, and dried. The yield of the extracted material amounted to 29 grams, 58 per cent of the weight of the starting material. The extracted substance was added to 600 cc. of cold fuming hydrochloric acid (d. 1.22 at 15° C.), and the mixture was well stirred and allowed to remain for 24 hours in a cool place (+8 to +10° C.). The reaction mixture was poured into 4 liters of ice water and filtered. The product was air-dried and treated with 300 cc. of fuming hydrochloric acid according to the experimental procedure indicated above. The reaction mixture was again poured into

[1] *Cellulosechem.*, **2**, 81 (1921).
[2] Marion, *Can. J Research*, **6**, 521 (1932).
[3] *J. Agr. Research* **29**, 523 (1924).
[4] Colorado Expt. Sta. Forestry Sec. Bull. 307 (1926).
[5] *Z Pflanzenkrankh.*, **38**, 257 (1928).

4 liters of ice water, filtered, washed with hot water, air-dried, and finally dried in vacuo at 56° C. over phosphorus pentoxide. Yield, 23 grams, 46 per cent of starting material. The product was dark brown.

Analysis (ash-free basis).—Found. C, 60.91, 60.97; H, 3.52, 3.68; OCH_3, 0.24, 0.26.

Chlorination.—Three grams of the material was suspended in 100 cc. of dry carbon tetrachloride, and a slow stream of dry chlorine was passed in for several hours until no more hydrochloric acid gas was evolved. The reaction product was filtered, washed with dry carbon tetrachloride, and dried in vacuo at 56° C., first over solid potassium hydroxide and then over phosphorus pentoxide. Yield, 3.89 grams. The product was amorphous and reddish-brown in color.

Analysis.—Found. Cl, 36.25, 36.54.

Bromination.—Two grams of the material was suspended in 25 cc. of dry carbon tetrachloride, and a few crystals of iodine were added, followed by 25 cc. of a 4 per cent solution of bromine in carbon tetrachloride. The mixture was refluxed on the boiling water bath for 3 hours. The reaction product was filtered off, washed with carbon tetrachloride, and dried in vacuo at 80° C., first over solid potassium hydroxide and finally over phosphorus pentoxide.

Analysis.—Found. Br, 29.92, 29.64.

Hydrolysis of the Bromo Derivative.—One gram of the bromo derivative was added to 100 cc. of a 5 per cent aqueous sodium acetate solution, and the mixture was boiled under a reflux condenser for one-half hour. The reaction mixture was filtered, the filtrate was acidified with nitric acid, and the bromine was precipitated with silver nitrate solution.

Analysis.—Found. Br, 21.29 (71.49 per cent of the total bromine in the original brominated substance).

Acetylation.—To 5 grams of the material 25 cc. of acetic anhydride and a few drops of concentrated sulfuric acid were added, and the mixture was heated on a boiling water bath, under a reflux condenser, for 2 hours. The reaction mixture was allowed to cool to room temperature and then poured into ice water. The reaction product was filtered off, washed with ice water, and dried in vacuo at 56° C., first over solid potassium hydroxide and then over phosphorus pentoxide.

Analysis.—Found. CH_3-CO, 25.0, 24.6; OCH_3, 0.20, 0.21.

The percentage acetyl was determined by the method described previously by Phillips.[1]

Methylation with Diazomethane.—To an ether solution of diazomethane containing about 1.8 grams of diazomethane, 4 grams of the material was added, and the reaction mixture was allowed to stand in a cool place for 3 days. It was then filtered off and dried in vacuo at 56° C. over phosphorus pentoxide.

[1] *Ind. Eng. Chem. Anal. Ed.*, **6**, 321 (1934).

Analysis.—Found. OCH_3, 16.07, 16.00.

Methylation with Dimethylsulfate.—Five grams of the material was dissolved in 100 cc. of 10 per cent sodium hydroxide solution, and to this solution was added, portionwise, 24 cc. of dimethylsulfate while the reaction mixture was stirred mechanically. When the reaction was over, the mixture was heated on the steam bath for 1 hour; it was then filtered, and the reaction product was remethylated by the procedure given previously. The product was washed with water and dried at 100° C.

Analysis.—Found. OCH_3, 21.97, 22.00.

The product obtained in the above experiment was methylated again with 24 cc. of dimethylsulfate and 15 cc. of 40 per cent sodium hydroxide solution. The reaction product was thoroughly washed with water and dried in vacuo at 56° C. over phosphorus pentoxide.

Analysis.—Found. OCH_3, 27.76, 27.50.

Nitration.—A nitrating solution was prepared by mixing 50 grams of nitric acid (d. 1.42) and 50 grams of sulfuric acid (d. 1.84). This solution was cooled to zero, and 5 grams of the material was added portionwise while the reaction mixture was well stirred. When all the material had been added, the reaction mixture was allowed to remain in a cool place (+8° C.) for 48 hours. It was then poured into ice water, and the orange-colored precipitate was filtered off and washed free of acid. The product was dried in vacuo at 56° C. over phosphorus pentoxide.

Analysis.—Found. N, 5.85, 5.87.

Alkali Fusion.—Fifty grams of potassium hydroxide and 25 cc. of water were put in a nickel crucible, and the mixture was heated at a temperature of approximately 100° C. until all the potassium hydroxide dissolved. To the alkaline solution was added, portionwise, a mixture of 10 grams of the material and 10 grams of zinc dust, and the reaction mixture was well stirred. The temperature of the mixture was gradually raised to 250° C. and maintained at this point for one-half hour. The temperature was finally raised to 310° C. and kept there for 15 minutes. The melt was allowed to cool, then dissolved in water, acidified with sulfuric acid, and distilled in a current of steam until the fresh distillate no longer reacted acid. The distillate was neutralized with sodium hydroxide and evaporated to dryness on the steam bath. The p-toluide of the sodium salt of the volatile acid was prepared according to the method given by Mulliken.[1] The p-toluide melted at 148.0° C. (corr.), and when mixed with some pure acet-p-toluide no depression in the melting point of the mixture could be observed. The volatile acid present was, therefore, acetic acid.

The solution remaining in the distilling flask from the steam distillation was filtered, and the filtrate was repeatedly extracted with ether. After

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[1] Mulliken, A Method for the Identification of Pure Organic Compounds, vol. I., p. 81. John Wiley and Sons, New York (1905).

removal of the ether a dark-brown sirup was obtained, and as no crystalline substance separated out after the sirup was allowed to stand for several days, the product was methylated by the addition of 25 cc. of a 10 per cent sodium hydroxide solution and 10 cc. of dimethylsulfate. The reaction mixture was cooled and extracted with ether. The ether extract consisted of a small quantity of gummy matter that had a rather pleasant odor, similar to veratrol. Nothing definite was isolated from this material. The alkaline solution, which had been extracted with ether, was acidulated with hydrochloric acid and again extracted with ether. This process removed a considerable amount of dark-brown amorphous material, which was dissolved in boiling water, decolorized with "Norit," and was then filtered and concentrated. Crystals separated out, and when purified by repeated crystallization from hot water, they melted at 181° C. (corr.). When mixed with some pure veratric acid, the melting point of the mixture was also 181° C. (corr.). The optical properties[1] of the crystals were found to be the same as those of veratric acid, although an exact determination of the refractive indices of veratric acid was found to be impossible. It is thus apparent that protocatechuic acid was obtained by the fusion of the lignin-like complex of *F. pini* with potassium hydroxide.

In another experiment in which the alkali fusion was conducted exactly as described above except that no zinc dust was used, oxalic acid was obtained (identified as oxal-p-toluide M. 275° C. corr.). The residue obtained from the ether extract of the acidified reaction product gave color reactions for protocatechuic acid, but it was not possible actually to isolate this acid.

Isolation of the Lignin-like Substance from the Sporophores of F. Pini with Methyl Cellosolve (ethylene glycol monomethyl ether).—Twenty-five grams of ground sporophores (extracted successively with a 1:2 alcohol-benzene solution and hot water and then dried), 250 grams of methyl cellosolve, and 25 cc. of hydrochloric acid (1+1) were mixed and heated together on the steam bath for 4 hours. The reaction product was filtered, the residual material was washed with hot methyl cellosolve, and the washings were added to the main filtrate. The weight of the residual material after drying amounted to 14.0 grams, 52.2 per cent of the weight of the starting material. The filtrate was concentrated to a small volume by distillation under reduced pressure, and the concentrated solution was poured into 2 liters of water that had been acidified with 5 cc. of concentrated hydrochloric acid. The precipitate was filtered off, washed with water, and was then boiled under a reflux condenser for one-half hour with 300 cc. of 2 per cent hydrochloric acid solution. The reaction product was filtered, washed with water, and then dissolved in a 2 per

[1] The optical properties of the crystals were determined by Mr. G. L. Keenan of the Microanalytical Laboratory of the Food and Drug Administration of this Department.

cent sodium hydroxide solution. This solution was filtered, and the filtrate was acidified with hydrochloric acid solution. The precipitate was filtered off, washed with hot water, and then dried, first in a desiccator and finally in vacuo at 56° C. over phosphorus pentoxide. Yield, 3.26 grams, 13.0 per cent of the weight of the starting material.

Analysis (ash-free basis).—Found. C, 59.96, 59.77; H, 4.23, 4.23; OCH_3, 3.88, 3.95 or 9.38, 9.55. Calculated as per cent—$O \cdot CH_2 \cdot CH_2$-OCH_3.

SUMMARY

A lignin-like substance was isolated from the sporophores of *F. pini* (Thore) Lloyd by the fuming hydrochloric acid method and by the methyl cellosolve method used for the isolation of lignin from lignified materials of seed plants. The material, unlike lignin, was found to be essentially free of methoxyl groups, although in some of its chemical reactions it resembled lignin.

CONCERNING THE DYER METHOD FOR THE IDENTIFICATION AND DETERMINATION OF VOLATILE FATTY ACIDS

By E. P. CLARK (Bureau of Entomology and Plant Quarantine) and FRED HILLIG (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

The procedure proposed by D. C. Dyer[1] for the identification and determination of volatile fatty acids is now used extensively.

The method is based on the principle that when a dilute solution of a volatile fatty acid is steam distilled at constant volume, there is, at any given stage, a definite and characteristic relationship between the quantity of acid in the flask and that in the distillate. Dyer, using a 500 cc. distillation flask and maintaining the volume of the liquid being distilled at 150 cc., defined the distillation constant as the percentage of acid in the distilling flask at the beginning of the operation that comes over in 100 cc. of distillate. When, under these conditions, a dilute solution of a pure acid, such as acetic or butyric, is distilled and the percentage of original acid in a given volume of distillate is plotted against this volume, a straight line is obtained, as indicated in Figure 3. The slopes of all the lines so obtained for the various acids are different and characteristic. For a mixture of acids a curved line is obtained, and the nature of its slope is, to some extent, indicative of the acids present. These properties are utilized for the identification and determination of the various acids of the group. Calculations involved in handling the data thus obtained are adequately treated by Dyer.

[1] *J. Biol. Chem.*, **28**, 445 (1917).

A critical study of the method, however, has revealed that the distillation constants of the various acids depend largely upon the size and design of the apparatus, as well as upon the rate of distillation. The values recorded by Dyer are obtained, therefore, only under the exact conditions used by him, and since the instructions given in his paper are inadequate, his results are not reproducible.

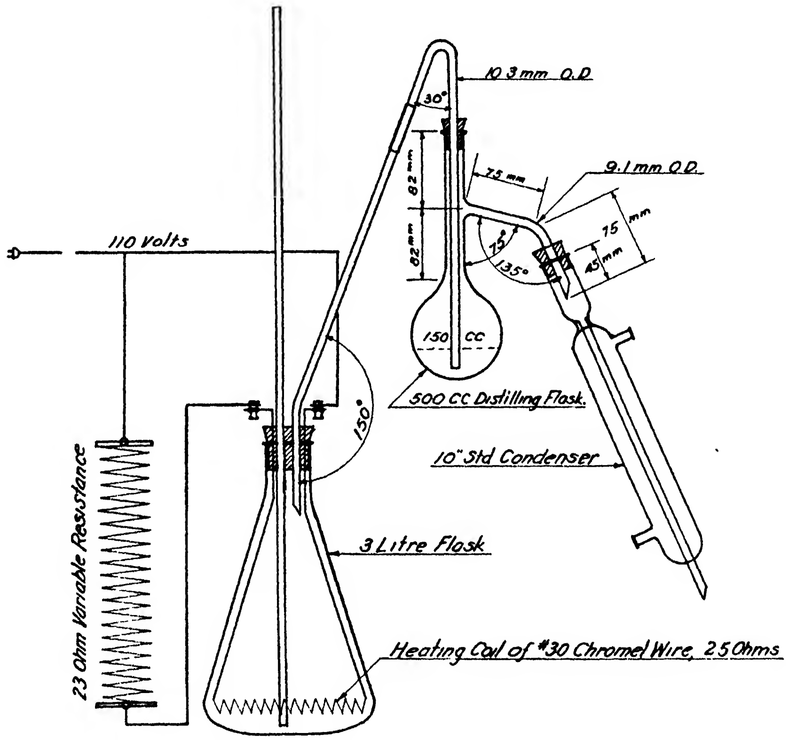

FIG. 1.—DISTILLATION APPARATUS

The object of this communication, therefore, is to call attention to these facts and suggest an apparatus and procedure by which consistent results may be obtained.

In the course of this study search was made for an assembly and a set of conditions that would reproduce the values obtained by Dyer, but this was unsuccessful. It was found, however, that the apparatus shown in Figure 1, when operated as directed below, gave consistent results with a number of assemblies. The distillation constants of the various volatile acids thus obtained differ from those reported by Dyer, but since they may be determined readily there seems to be no reason why new values may not be used. In fact, it seems logical for an analyst in this field to use his own assembly for determining the constants of the particular

acids in which he is interested. This does not affect the general principle of the method, and would give more accurate results.

APPARATUS AND PROCEDURE

The apparatus shown in Figure 1 is for the most part self-explanatory. Steam is generated by the electrically operated boiler, and accurately and

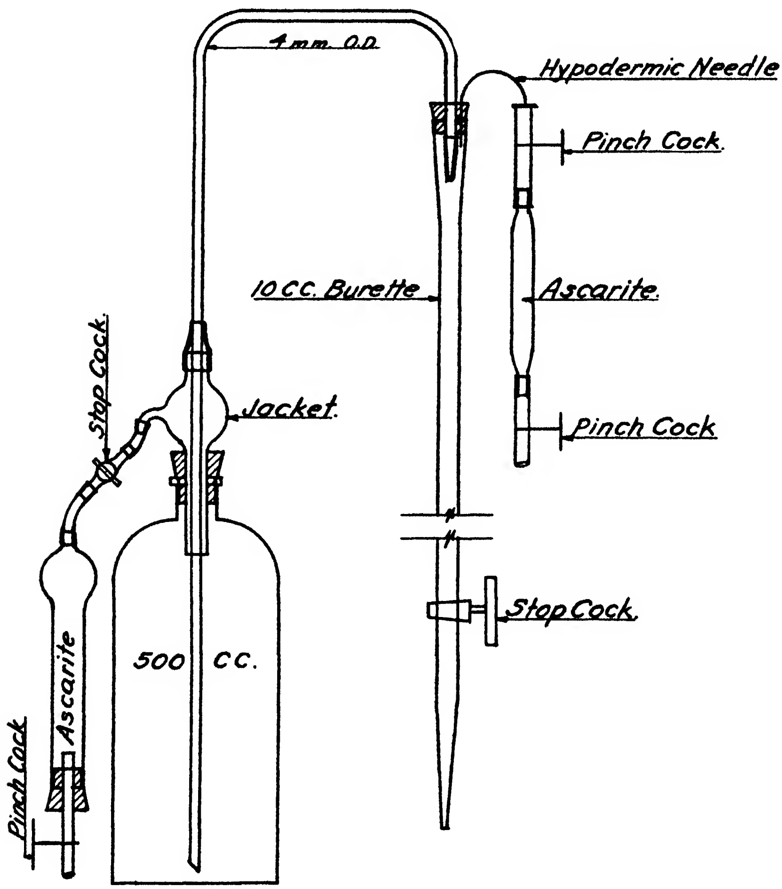

FIG. 2.—TITRATION APPARATUS

smoothly controlled by means of the rheostat. The liquid in the distilling flask, which contains from 1 to 100 mg. of free acid, is maintained at 150 cc. (marked on the flask), while the rate of distillation is so regulated by the flow of steam and a small flame under the flask that 100 cc. of distillate is collected in 30 minutes.

The distilled acids are titrated with standard barium hydroxide solution. Phenolphthalein is used as an indicator. The strength of the standard alkali used should range from 0.01 to 0.1 N, depending upon the

quantity of acid to be titrated. (These standard solutions should be preserved in paraffined bottles and protected from air by the device shown

TABLE 1.—*Distillation constants and rate of distillation of several common volatile fatty acids*

(The figures under each acid represent the percentage of the acid in the flask at the beginning of the distillation that comes over in the indicated volume of distillate. The first 100 cc. fraction is the distillation constant.)

DISTILLATE	FORMIC	ACETIC	PROPIONIC	n-BUTYRIC	ISO-BUTYRIC
cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
25	6.0	10.4	19.0	28.1	38.7
50	12.0	19.3	34.0	48.1	62.2
75	17.4	27.5	47.0	62.5	76.6
100	*23.0*	*34.7*	*56.8*	*72.8*	*85.6*
200	40.5	57.2	81.3	92.45	97.82
300	54.0	71.7	92.0	97.90	99.67
400	64.1	81.1	96.45	99.42	
500	72.1	87.5	98.45	99.84	
600	78.4	91.80	99.34		
700	83.3	94.55	99.72		
800	87.0	96.42	99.87		
900	90.0	97.65			
1000	92.2	98.43			

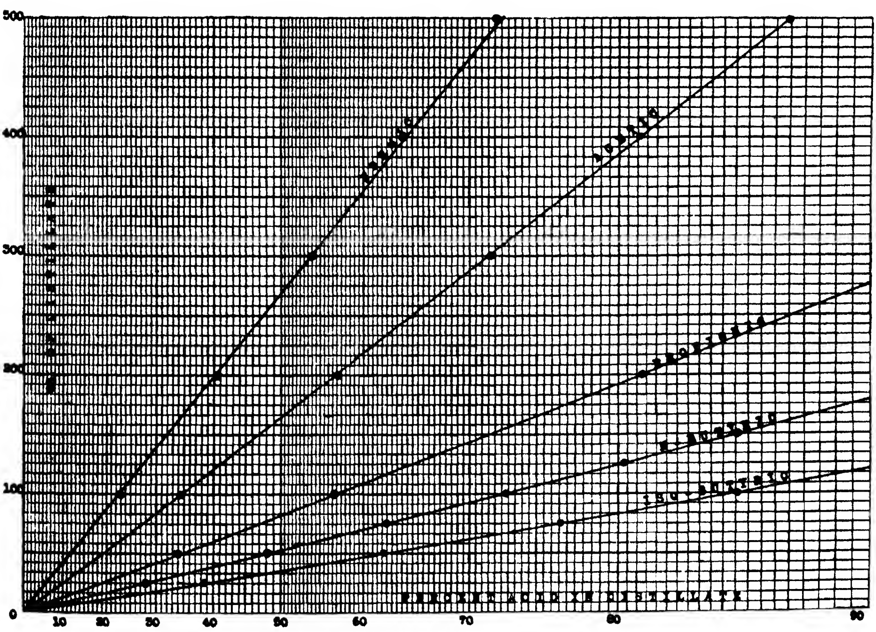

FIG. 3.—RATES OF DISTILLATION OF SEVERAL VOLATILE FATTY ACIDS, AS DETERMINED BY THE MODIFIED DYER METHOD

in Figure 2, or a similar one.) The titrations are carried to a definite end point by comparison with the color of an equal volume of standard (*p*H 8.6) buffered solution and indicator contained in a stoppered flask the same size as that used in the titration. Titration blanks are determined with the same volume of water freshly distilled from the apparatus. When these precautions are taken, the results are all that could be desired.

In Table 1 are given the distillation constants (the value for the first 100 cc.) and rates of distillation of a few of the more common volatile acids as determined by the above procedure.

Figure 3 is a graphical representation of some of these data.

A CHEMICAL PROCEDURE FOR EVALUATING SPOILAGE IN CANNED FISH, ESPECIALLY SALMON AND TUNA FISH

By FRED HILLIG (Food and Drug Administration) and E. P. CLARK (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

The problem of chemically detecting and estimating the degree of spoilage in canned fish has been the subject of numerous investigations. While this work has been only partially successful the facts that have emerged, when critically analyzed and supplemented by further research, have led to a practical solution of the problem in so far as its application to salmon and tuna fish is concerned. Preliminary work also indicates that the principles involved are generally applicable to other fish and to various types of packs.

The purpose of this paper is to outline the facts thus far developed and to present a procedure for solving the problem according to these findings.

It was found that canned fish prepared from the freshest possible raw material normally contains small quantities of volatile fatty acids, and that the greater part of these is formic and acetic. When spoilage begins the quantity of these acids increases, and at the same time traces of higher members of the series appear. As decomposition continues there is a progressive increase in volatile fatty acids, with the higher members of the series constituting a larger and larger proportion of the acid mixture. The identity of all the higher members of the series has not been determined, but it was found that in the case of tuna fish the highest member of the series is normal butyric acid, while in the case of salmon it is isobutyric acid.

After preliminary work indicated the presence of these acids in all samples investigated, their identification in material representing various stages of decomposition was undertaken. The method employed was that

of Dyer.[1] An outline of the procedure is presented, in which there was used as an illustration an experimental pack of canned tuna fish from raw material that was distinctly objectionable in odor, though not in an advanced stage of decomposition.

A quantity of the volatile fatty acids equivalent to ca. 75 cc. of 0.1 N acid was obtained by steam distilling a clarified water extract (see under method) of the fish. These acids were neutralized, concentrated to dryness, dissolved in a small quantity of water, transferred to a 100 cc. distilling flask containing 25 grams of crystalline $MgSO_4$ and adjusted to a volume of 50 cc. The acids were then steam distilled. Under these conditions practically all the acids were in the first 300 cc. of distillate. They were accurately titrated, evaporated to about 100 cc, transferred to a Dyer assembly, and steam distilled according to the modified procedure referred to above.

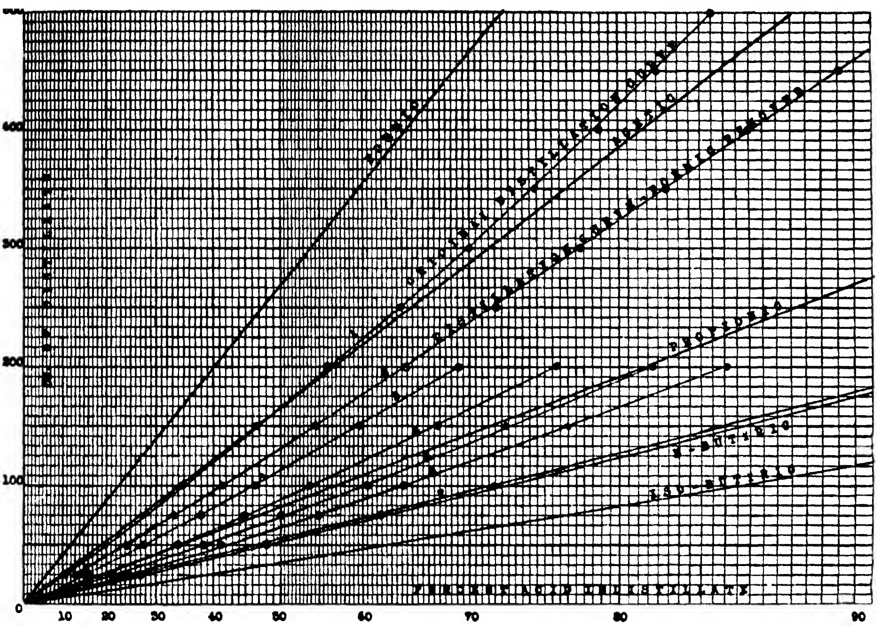

FIG. 1.—FRACTIONATION OF VOLATILE FATTY ACIDS FROM TUNA FISH, CODE B-3.

The distillation curve obtained (Curve 1, Figure I) indicates the presence of formic, acetic, and a higher member (or members) of this series of acids. These conclusions follow from an examination of Figure I. The original distillation curve starts below the acetic acid line, intersects it, and approaches the formic acid line. Because the curve intersects the acetic acid line, formic acid and one or more acids higher in the series than acetic acid are indicated, while in general the close proximity of the curve to the acetic acid line indicates the presence of this acid.

The neutralized acids in the several portions of distillate used to obtain Curve I were combined and evaporated to 50 cc. The formic acid was then destroyed with HgO and a new distillation curve (Curve 2) was obtained with the remaining acids. The new curve lies below the acetic acid line but in close proximity to it, thus again indicating acetic acid. However, as the curve lies *below* the acetic acid line a higher member of the series is also indicated. The first 100 cc. fraction of the distillate ob-

[1] *J. Biol. Chem.*, 28, 445 (1917). While the principles involved in this method are valid, the procedure required standardization. This phase of the work is reported upon separately (see p. 684).

TABLE 1.—*Analysis of canned salmon*

(The formic acid and volatile acid numbers are indicative of the degree of spoilage. The code numbers designate the grade of fish. Code I is prime fish, and the other code numbers progressively indicate spoilage. The various grades were experimental packs, and their classification was verified organoleptically.)

CAN NO.	CODE 1		CODE 2		CODE 3		CODE 4	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./100 g.	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*
1	0.68	9.3	0.68*	13.0*	1.69*	24.0	3.90*	44.8*
	0.68	9.3	0.91	12.8	1.82	24.4	4.12	44.5
2	0.52	9.5	0.78*	13.3*	2.60	26.2	4.48*	58.3
	0.58	9.4	0.84	13.1	2.50	25.6	4.42	57.6
3	0.39	9.8	0.72*	14.6	3.25	26.7	6.07	57.9
	0.52	9.8	0.81	14.6	3.28	26.2	6.17	58.8
4	0.52	10.1	1.01	14.9	2.53	29.6	8.15	69.3
	0.58	10.4	1.08	15.1	2.79	29.6	7.73	69.0
5	0.46	10.7	1.14	17.2	2.69	30.6	7.05	76.0
	0.49	10.6	1.10	17.0	2.53	30.6	7.08	76.2
6	0.49	11.4	1.53	18.1	3.86	37.0	8.09	78.7
	0.68	11.2	1.53	17.6	3.78	36.7	7.83	78.7
7	0.58	11.2	1.27	20.5	3.54	36.8	7.01	79.3
	0.65	11.5	1.36	20.8	3.90	37.1	7.23	79.9
8	0.78	11.3	1.56	21.1	4.81	41.3	7.63	87.3
	0.78	11.5	1.56	21.5	4.61	41.4	7.99	88.3
9	—	12.8	1.85	22.4	4.76	43.8	11.46	98.2
	0.84	12.8	1.75	22.4	5.00	44.2	11.46	99.9
10	0.97	13.1	1.53	22.8	5.19	50.1	10.45	101.1
	1.07	13.9	1.46	22.4	4.57	49.5	10.26	100.6
11	—	12.0	—	18.1	—	43.2	—	59.1
		12.2		17.7		43.0		58.7
12	—	11.3	—	17.8	—	53.4	—	82.1
		11.3		17.3		53.7		82.1
13	—	13.7	—	17.8	Av. 3.49	36.9	—	67.5
		13.7		17.3	Max. 5.19	53.7		67.4
					Min. 1.69	24.0		

TABLE 1.—*Continued*

CAN NO.	CODE 1		CODE 2		CODE 3		CODE 4	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg/100 g.	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*
14	—	13.3	—	18.1			—	93.0
		13.2		18.2				94.5
15	—	13.2	Av. 1.22	17.8			—	70 1
		12.9	Max. 1.85	22.8				70.6
			Min. 0.68	12.8				
16	—	11.5					—	58.8
		11.1						58 5
17	—	11.7					—	78.3
		11.4						76 3
18	—	12.0					—	59 4
		11.8						61.0
19	—	13.6					—	89 8
		13.3						92 7
20	Av. 0.65	11.7					—	86.3
	Max. 1.07	13.9						84.7
	Min. 0.39	9.3						
							Av. 7.43	75 0
							Max. 11.46	101.1
							Min. 3 90	44 5

* Indicates overlapping into next lower code.

tained in preparing Curve 2 was then distilled as before; the various fractions were titrated and the values were plotted as Curve 3. This process, repeated several times, gave the succeeding curves. Since Curve 7 coincides with that of n-butyric acid and a repetition of the process did not change the slope of the line, it follows that the highest member of the series of acids present is normal butyric. It does not, however, prove the presence or absence of propionic acid.

This procedure was then followed in investigating the components of the mixture of volatile fatty acids obtained from a number of experimental packs of salmon and tuna fish.

A critical examination of the extensive data accumulated shows that for practical diagnostic purposes it is not necessary to determine the entire quantity of acid in a sample, nor is it necessary to identify all the components of the mixture. What is essential is to determine accurately *a definite proportion* of the total acids, which has been called the "volatile acid number" and *the quantity of formic acid in this fraction,* designated as the "formic acid number."

(The same considerations as those given for salmon in Table 1 apply to this series.)

CAN NO.	CODE B1		CODE B2		CODE B3		CODE B4		CODE B5	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./100 g.	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*
1	1.20	18.3	2.21	29.9	6.72	74.2	18.60	176.2	21.20*	343.7*
	1.20	18.1	2.25	29.8	6.45	74.0	19.44	178.5	21.00	344.9
2	1.46	20.7	2.59	31.8	7.57	79.7	14.81*	194.8	24.35	449.2
	1.62	21.0	2.50	31.9	7.76	79.0	15.19	197.2	24.12	447.9
3	—	21.0	2.59	34.1	8.12	81.6	14.81*	197.8	16.36*	451.2
	1.43	21.1	2.59	33.9	7.95	80.8	15.01	200.2	16.43	453.5
4	1.46	22.4	3.05	34.4	8.38	88.7	12.37*	205.1	25.84	459.5
	1.46	21.8	2.79	34.4	8.38	89.9	12.21	206.1	25.75	461.2
5	1.49	22.3	3.25	34.2	8.15	95.6	19.19	213.5	18.60*	504.5
	1.56	22.0	3.25	35.5	8.28	97.1	18.86	216.5	18.80	512.2
6	1.56	23.6	3.18	40.5	11.20	105.5	15.13*	226.3	18.05*	574.8
	1.56	23.4	3.21	40.5	10.39	104.7	15.45	228.1	18.28	582.7
7	1.75	23.7	3.28	41.9	14.38	114.2	18.83	242.3	18.86*	584.4
	1.85	24.3	3.41	41.6	14.06	113.2	19.37	245.6	18.96	587.7
8	1.36	24.3	3.93	47.3	14.61	117.5	22.60	248.4	16.40*	583.3
	1.46	24.0	3.73	47.1	14.58	116.9	22.24	246.4	16.50	581.1
9	1.80	24.3	4.94	55.5	16.14	129.5	15.42*	277.7	22.73	590.4
	1.82	24.0	4.68	55.2	16.14	122.0	15.42	279.1	22.30	589.7
10	1.88	26.2	4.87	57.2	16.78	132.3	17.76	348.0	22.93	609.0
	1.80	26.0	5.13	58.6	16.43	129.2	18.15	349.7	22.66	607.7
11	—	—	—	36.6	—	76.9	—	317.3	Av. 18.60	515.9
		25.6		36.3		75.9		315.7	Max. 25.84	609.0
									Min. 16.36	343.7

TABLE 2.—*Continued*

CAN NO.	CODE B1		CODE B2		CODE B3		CODE B4		CODE B5	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./100 g.	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*
12	—	25.0	—	63.7	—	96.7	—	227.4		
		23.7		63.1		93.8		226.8		
13	—	24.2	—	39.0	—	98.3	—	253.7		
		23.1		38.3		98.2		250.1		
14	—	23.2	—	45.6	—	102.0	—	321.7		
		22.5		45.2		102.0		320.0		
15	—	19.7	—	40.4	—	86.1	Av. 17.04	243.2		
		18.8		40.6		86.6	Max. 22.60	349.7		
							Min. 12.21	176.2		
16	—	27.2	Av. 3.37	41.5	—	135.5				
		26.4	Max. 5.13	58.6		135.2				
			Min. 2.21	29 9						
17	—	25.5			—	108.1				
		25.5				106.9				
18	—	25.4			—	116.7				
		25.1				116.3				
19	—	24.3			—	124.2				
		23.9				123.3				
20	—	23.3			—	112.5				
		23.0				111.1				
	Av. 1.56	23.3			Av. 11.12	104.4				
	Max. 1.88	27.2			Max. 16.78	135.2				
	Min. 1.20	18.3			Min. 6.45	74.0				

* Indicates overlapping into next lower code.

In brief, the method proposed consists of suspending a 50 gram sample of canned fish in water, adding phosphotungstic acid to the mixture, removing the solids by filtration, and steam distilling the resulting liquid according to the modified procedure referred to previously. The distillate is collected in two 100 cc. portions. These are titrated separately, as proposed by Dyer, after which they are united and the quantity of formic acid in the combined portions is determined. These data are sufficient to determine the extent of spoilage in canned fish as is well shown by the information contained in the tables. The various codes represent experimental packs. Code I represents raw material of unquestioned freshness, while the succeeding codes represent progressive stages of spoilage up to and including badly tainted fish.

TABLE 3.—*Analysis of canned mackerel*

CAN NO.	CODE 1		CODE 2		CODE 3	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./100 g.	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*	*mg./100 g.*	*cc. N/100 per 100 g.*
1	0.82	12.3	1.85	21.5	3.60	48.7
	0.91	12.7	1.91	21.0	3.54	48.6
2	0.68	11.7	1.91	23.3	4.03	58.7
	0.75	11.4	1.79	22.8	3.83	59.3
3	0.94	12.5	1.76	19.8	3.25	51.2
	0.91	12.3	1.85	19.6	3.28	51.8
Av.	0.84	12.2	1.85	21.3	3.59	53.1
Max.	0.94	12.7	1.91	23.3	4.03	59.3
Min.	0.68	11.4	1.76	19.6	3.25	48.6

With modification, the method may be used on sauce-packed fish. Preliminary work has shown that the volatile acid number on such material (sardines) is of little value because of the excessive quantities of acids in the sauce (essentially acetic acid). However, if the formic acid number is determined, the method becomes valuable as an index of the condition of any sample under investigation.

The method follows:

METHOD

Volatile Acid Number.—Pass the entire contents of a can of fish through a meat chopper three times and thoroughly mix the material after each grinding. Weigh 50 grams of this material into a 250 cc. beaker, stir to a uniform suspension with 100 cc. of water, and transfer quantitatively to a 250 cc. volumetric flask. Add 15 cc. of 2 N H_2SO_4, mix well, and add 15 cc. of 20% phosphotungstic acid solution (with tuna fish use 25 cc. of each reagent). Dilute the mixture to 250 cc., *shake*

vigorously, allow to stand 5 minutes, and filter through a folded filter paper. Transfer 150 cc. of the filtrate to the distillation flask of the modified Dyer distillation assembly described by Clark and Hillig (p. 684), add 0.5 cc. of 50% H_2SO_4 (with tuna fish use 1 cc.), and distil at constant volume at a uniform rate of 200 cc. per hour until two 100 cc. portions have been collected. Titrate each portion separately with 0.01 *N* $Ba(OH)_2$ solution, using phenolphthalein as an indicator, until the color matches that of an equal volume of a *p*H 8.6 buffer solution with the same quantity of indicator present and contained in the same sized flask as that used in the titration. From the acid in both 100 cc. portions, after subtracting the titration blank, calculate the number of cc. of 0.01 *N* acid that would be obtained from 100 grams of fish. This is called the "volatile acid number."

Formic Acid Number.—Combine the two 100 cc. portions of titrated distillate obtained above, add 2 drops of saturated $Ba(OH)_2$ solution, and evaporate the resulting liquid to dryness on a steam bath. Add 5 cc. of water to the residue and 1 cc. more of normal HCl than is necessary to liberate the volatile acids. Filter through a small paper into a 125 cc. ground joint Erlenmeyer flask, and wash the paper with water in such a manner that the total filtrate will equal approximately, but not exceed, 30 cc. Add 5 cc. of 10% aqueous $NaC_2H_3O_2$ solution, 5 cc. of 5% NaCl solution and 10 cc. of 5% $HgCl_2$ solution. Place the flask, with a ground joint air condenser, on a steam bath and allow the liquid to react for 2.5 hours. Transfer the precipitate of calomel to a tared Pregl filter tube provided with a mat of asbestos about 2 mm. thick.[1]

Wash the precipitate with water, followed by alcohol, and dry for 0.5 hour at 100°. After the tube cools, weigh the calomel, using as a tare another tube prepared and treated in exactly the same manner as that containing the precipitate. The weight of the precipitate multiplied by 0.0975 gives the weight of formic acid in the sample. Three and one-third times this value gives the formic acid in 100 grams of fish. This value has been termed the "formic acid number."

SUMMARY

A method for the evaluation of spoilage in canned fish, especially salmon and tuna fish, is proposed. The procedure is simple and direct, and yields accurate consistent results.

SOME NOTES ON THE STABILITY OF DITHIZONE SOLUTIONS

By P. A. CLIFFORD (U. S. Food and Drug Administration, Washington, D. C.)

The purity and stability of dithizone solutions have engaged the attention of practically every investigator using the reagent in the determination of the various dithizone metals. As preparation of fresh dithizone solutions or frequent restandardization is bothersome, methods for their stabilization should be valuable. Therefore it is proposed to outline the procedures that the writer has used most successfully in the preservation of both chloroform and carbon tetrachloride solutions of dithizone with special reference to the "mixed-color" dithizone method for lead.[2]

[1] For the construction of the necessary apparatus and manipulative details see Pregl. Quantitative Organic Microanalysis, Translated by Fyleman, Blakeston's Sons and Co., Philadelphia. (1930).
[2] Clifford and Wichmann, *This Journal*, 19, 130 (1936).

The photometric procedures specified in the mixed-color method for lead are based upon the measurement of the light absorption of the red phase of the color mixture at a point (around 510 mμ) where the absorption of the green dithizone itself is small. Because it is small, variations in the concentration of free dithizone must be considerable to influence the results appreciably. The absorption of the green phase, however, is not insignificant[1] and accounts for the fact that standard curves do not begin at the origin. Thus, standard curves prepared from a chloroform solution during the course of deterioration will parallel the original curve closely, but will fall successively below it. In other words, the value of the intercept, depending upon the absorption of the green phase, will fall, but the slope will not change to any appreciable extent.

On the other hand, Winkler's mixed-color method for mercury[2] applies the converse principle and measures the absorption of the mixed colors at about 610 mμ. Here, values represent almost entirely the absorption of the uncombined dithizone in the color mixture rather than that of the metal complex. Any appreciable variation in dithizone strength (at this point where dithizone solutions absorb strongly) causes corresponding variation in results, and the stability of the standard dithizone solution becomes more important.

Because chloroform and carbon tetrachloride are used almost exclusively in dithizone methods, experiments were confined to these two solvents.

PERMANENCY OF CHLOROFORM SOLUTIONS

In previous work the writer has used fresh, redistilled U.S.P. chloroform as the solvent for standard dithizone solutions. When these solutions were kept out of direct sunlight at ordinary room temperature, deterioration during any cool weather month was usually not sufficient to affect the accuracy of lead results appreciably. Deterioration during the summer months was more rapid. Thus, a standard dithizone solution stored at room temperature during the cooler spring months still yielded values accurate to 2 per cent of the top of the lead range (when the original factor was used) at the end of 40 days, while a solution stored during the month of July at room temperature gave values in error by about 10 per cent, mainly due to the lowering of the value of the intercept. In the latter case the dithizone concentration, measured photometrically with an orange filter centering at about 610 mμ, had dropped to approximately 75 per cent of its original value. (This is about the optimum point for the measurement of dithizone decomposition, because (1) here absorption of dithizone is maximum, and (2) absorption of the yellow phase resulting from dithizone oxidation is minimum.) A portion of the same solution stored during July in the icebox showed no appreciable loss in concentration.

[1] Laug, *This Journal*, 21, 481 (1938).
[2] *This Journal*, 21, 220 (1938).

The primary cause of decomposition of dithizone in chloroform solutions appears to be heat, and the influence of ordinary diffused daylight, while appreciable, is almost negligible by comparison. For example, two portions of the same chloroform solution of dithizone were stored in the same room; one was exposed to light, and the other was held in a darkened cabinet. The course of decomposition was followed photometrically over a period of 65 days, at the end of which time the concentration of the exposed solution had dropped to 65 per cent, while the solution stored in the dark had fallen to 71 per cent, of its original strength.

Direct sunlight, on the other hand, will rapidly bleach a chloroform solution of dithizone. The effect is upon the solvent rather than upon the dissolved dithizone. To demonstrate this point, a quantity of chloroform was divided, and one portion kept in the dark while the other portion was exposed to direct sunlight for 2 hours. The exposed and unexposed portions were then used to make up dithizone solutions of identical concentration. The solution made from the exposed chloroform bleached completely within 2 hours, while the other showed no change.

Decomposition of chloroform is indicated by the appearance of free chloride and a phosgene odor. Chloroform giving a positive test for chloride is unsuited for use in standard dithizone solutions.

The reclamation process of Biddle[1] has proved to be of great value in the recovery of chloroform from spent dithizone solutions, and even in improving the quality of fresh U.S.P. chloroform. However, certain precautions should be observed. It is essential that the alcohol needed as a preservative be replaced as quickly as possible after the distillation, because it was noted that chloroform that had stood 2 hours before the alcohol was added back, would oxidize dithizone instantly. Hence, there was adopted the practice of measuring the volume of acid-washed chloroform to be distilled and adding 1 per cent of this volume of alcohol to the flask in which the distillate is to be received. As a further precaution the receiver should be swirled several times during the distillation to mix the contents. It was likewise noted that absolute alcohol, freshly redistilled from solid potassium hydroxide, was much superior to ordinary 95 per cent alcohol as a preservative. Further precautions involve the use of all-glass apparatus and a water bath instead of a direct flame to heat the distilling flask.

Methods of stabilizing chloroform solutions of dithizone, involving the use of hydroxylamine, sulfurous acid, or sodium thiosulfate, proved of little value. It is thought that the procedure of Hubbard[2] is not completely effective because the preserving alcohol is partially removed in the washing process. Therefore the writer tried hydroxylamine hydrochloride as a preservative (while still retaining the alcohol), by using a 1 per cent volume of purified absolute alcohol saturated with this salt, instead of

[1] *Ind. Eng. Chem. Anal. Ed.*, **8**, 99 (1936).
[2] *Ibid.*, **9**, 493 (1937).

alcohol alone, in Biddle's reclamation process, but no advantage was noted. Overlaying dithizone solutions of reclaimed chloroform with aque-

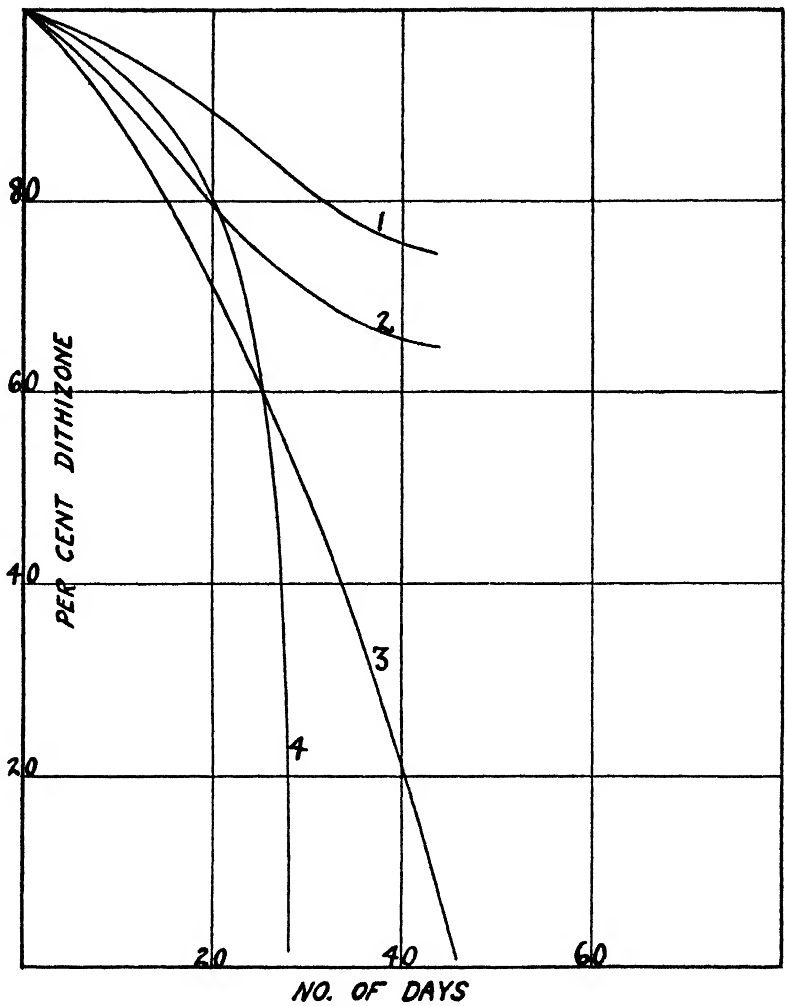

FIG. I.—1. DITHIZONE SOLUTION IN $CHCl_3$, PURIFIED ACCORDING TO BIDDLE; 2. DITHIZONE SOLUTION IN SAME $CHCl_3$ AS 1, FURTHER TREATED ACCORDING TO HUBBARD; 3. DITHIZONE SOLUTION IN UNPURIFIED, UNTREATED U.S.P. $CHCl_3$; 4. DITHIZONE SOLUTION IN PURIFIED $CHCl_3$; 1% OF ABSOLUTE ALCOHOL SATURATED WITH HYDROXYLAMINE HYDROCHLORIDE USED AS PRESERVATIVE.

ous solutions of hydroxylamine or sodium thiosulfate or the use of sulfur dioxide as an aqueous solution or by partially saturating the chloroform solution with the dry gas likewise offered no advantage.

In all cases, results reported in this paper are based upon a study of decomposition rates followed photometrically with an orange filter. Solutions were stored at room temperature, exposed to ordinary diffused daylight, in well-corked, white glass flasks. Results are charted in Figures I and II. (Data in these two charts are not comparable as they represent studies made at different times with different batches of chloroform.)

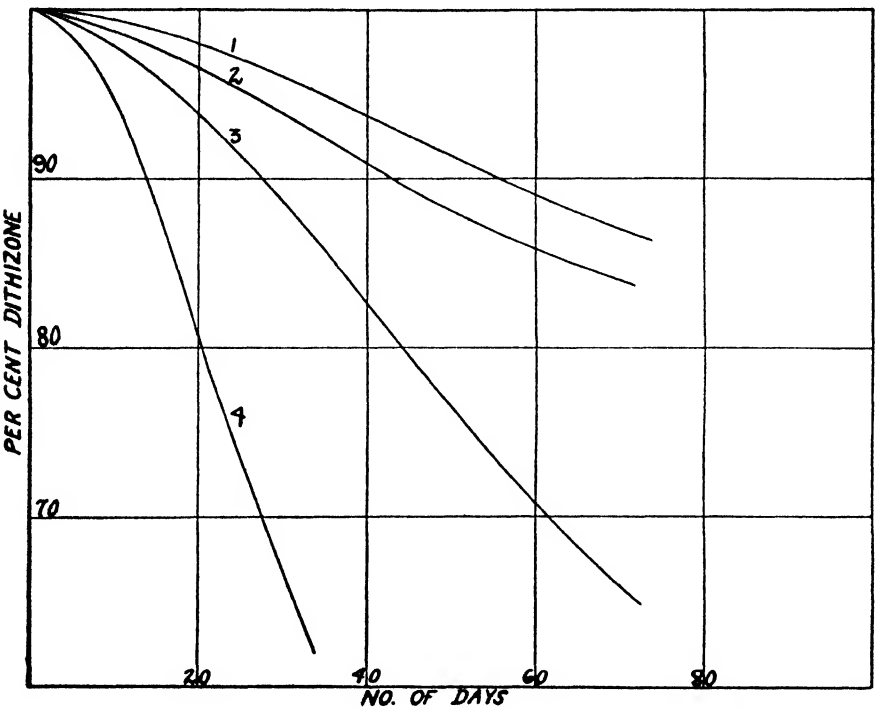

FIG. II.—1. DITHIZONE SOLUTION IN $CHCl_3$, PURIFIED ACCORDING TO BIDDLE; 1% PURIFIED ABSOLUTE ALCOHOL; 2. DITHIZONE SOLUTION IN SAME $CHCl_3$ AS 1, STORED UNDER 0.5% HYDROXYLAMINE HCl SOLUTION; 3. DITHIZONE SOLUTION IN $CHCl_3$, PURIFIED ACCORDING TO BIDDLE; 1% 95% ALCOHOL; 4. DITHIZONE SOLUTION IN SAME $CHCl_3$ AS 1, STORED UNDER 0.5% HYDROXYLAMINE HYDROCHLORIDE SOLUTION PREVIOUSLY NEUTRALIZED TO pH 7.5.

The writer's experience indicates that the most stable chloroform solutions of dithizone are made from fresh U.S.P. chloroform that has been further purified by Biddle's process, with purified absolute alcohol as the preserving agent. Standard solutions are made up in considerable quantity with carefully-purified dithizone,[1] and stored in a dark room at 40° F. Portions are withdrawn as needed and may be kept at room temperature for several days before becoming unsuitable for use. Thus, a chloroform

[1] *Methods of Analysis, A.O.A.C.*, 1935, 378.

solution of dithizone of 4 mg./1 concentration, stored for a period of 6½ months, showed a decrease in dithizone strength of only 5 per cent, and negligible change in position and slope of its standard curve. It is unnecessary to purify in a special manner fresh chloroform that is to be used in making dithizone solutions for simple extraction work.

PERMANENCY OF CARBON TETRACHLORIDE SOLUTIONS

Possible advantages of carbon tetrachloride over chloroform solutions of dithizone in the mixed-color method for lead have been pointed out.[1] As a rule, carbon tetrachloride solutions are more unstable than chloroform solutions because they are more directly affected by light and heat. Biddle's process was likewise found to be applicable to the reclamation of used carbon tetrachloride and, as with chloroform, greatly improved the quality of unused C.P. carbon tetrachloride. No alcohol is used with carbon tetrachloride.

Sulfur dioxide has been recommended as a preservative for carbon tetrachloride solutions of dithizone by Fischer and Leopoldi,[2] who overlay their solutions with sulfur dioxide water. It was found that partially saturating an old and partly decomposed carbon tetrachloride solution of dithizone with the dry gas would convert the oxidized dithizone to its original green form. A practical test of dithizone purity consists in "stripping" its carbon tetrachloride solution with dilute ammonia-potassium cyanide mixture, when the dithizone will pass into the aqueous phase as the orange-colored salt, and impurities, chiefly oxidized dithizone, will impart a residual yellowish to brownish color to the solvent layer. After being gassed for one hour with sulfur dioxide under slight pressure, a partially decomposed dithizone solution was observed to "strip" water-white, which indicated complete reconversion of oxidized dithizone. The photometric readings, meanwhile, had risen from relative density values (proportional to dithizone concentration) of 64 to 87. The gassed solution was then stored at room temperature, and photometric readings were observed to remain at this level for four days. They then began to decline, until at the end of 21 days they had fallen to a value of 74. At this point the solution was re-gassed, but the readings did not rise as before, and after 30 days the dithizone solution had fallen to about its original concentration. It appeared to be more practical to use sulfur dioxide in the form of its aqueous solution, as much of it is absorbed by carbon tetrachloride when the dithizone solutions are treated directly, and it was feared that the standard pH conditions of the color development would be disturbed by the dissolved gas. Dilute aqueous solutions were found to have a similar effect, the readings increasing to a maximum within a day or two (provided the dithizone was noticeably impure at the start), then

[1] Referee's Report on Lead, *This Journal*, 21, 212 (1938).
[2] *Wiss. Veröffent. Siemens Konzern*, 12, 44 (1933).

slowly falling. Optimum strength of the sulfur dioxide water is 0.1–0.01 *M* as appears from the decomposition study charted as Figure III. These solutions were stored in white flasks at room temperature and covered with a one-tenth volume of sulfur dioxide water.

Carbon tetrachloride solutions of dithizone preserved under 0.1 *M* sulfur dioxide and stored in the cold and in the dark appear to retain their strength almost indefinitely. Solutions stored in the dark at 40° F. for

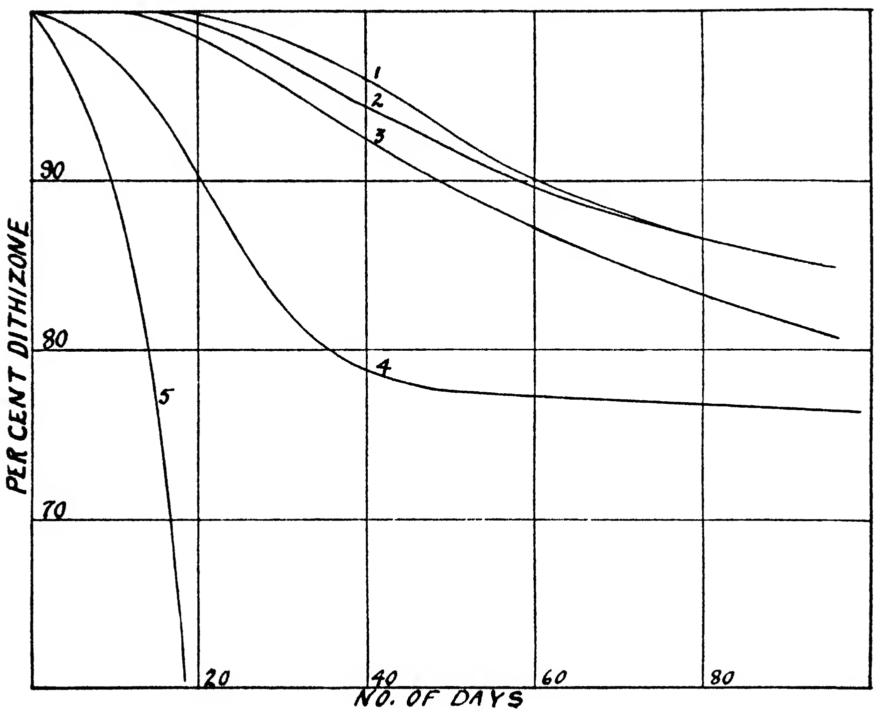

FIG. III.—1. DITHIZONE SOLUTION IN PURIFIED CCl_4 STORED UNDER 0.1 *M* SO_2 WATER; 2. DITHIZONE SOLUTION IN PURIFIED CCl_4 STORED UNDER 0.01 *M* SO_2 WATER; 3. DITHIZONE SOLUTION IN PURIFIED CCl_4 STORED UNDER 1.0 *M* SO_2 WATER; 4. DITHIZONE SOLUTION IN PURIFIED CCl_4 STORED UNDER 0.001 *M* SO_2 WATER; 5. DITHIZONE SOLUTION IN PURIFIED CCl_4 (CONTROL; NO SO_2 WATER).

nine months showed no change except a very slight (less than 1 per cent) increase in concentration, due undoubtedly to evaporation of the solvent through the aqueous layer and the stopper. Standard curves prepared at the beginning and end of the nine months' storage period were practically identical. The presence of a small amount of sulfur dioxide in the standard carbon tetrachloride solution of dithizone apparently produces no disturbing effects in the lead method.

A convenient storage vessel is a large, narrow-mouthed Pyrex bottle

fitted with a 2-holed cork stopper. An outlet tube extends through the aqueous layer to the bottom of the bottle and is bent to form a jet, which can be closed by a glass stopcock, on the outside. The other hole admits a short piece of glass tubing, which can be closed by means of a short piece of rubber tubing and pinchclamp. The desired amount of dithizone solution can be drawn off by blowing through the short tube as with a wash bottle. Only enough for one day's supply should be withdrawn. The writer has used sulfur dioxide water equal in volume to about one-tenth that of the carbon tetrachloride to be preserved. It is made by bubbling the washed gas into carefully redistilled water and determining the solution's strength by titration. (A convenient indicator for an acidimetric titration is bromphenol blue, and it should be remembered that with this indicator aqueous solutions of sulfur dioxide titrate with sodium hydroxide as a monobasic acid.) When stored under sulfur dioxide water in the cold, about 2 weeks should be allowed for new carbon tetrachloride solutions to stabilize, and standard curves should not be run before this time. It is also convenient to prepare and preserve in like manner a strong stock solution of dithizone in carbon tetrachloride of about 400 mg./liter strength. This solution can be diluted down to standard strength with purified carbon tetrachloride as needed. The efficiency of sulfur dioxide also suggests ways to prepare very pure dithizone.

Solutions of hydroxylamine hydrochloride had no value as preservatives when used to overlay carbon tetrachloride solutions of dithizone.

The reasons why sulfur dioxide is efficient as a preservative with carbon tetrachloride solutions and not with chloroform solutions of dithizone are a matter of conjecture. According to H. Fischer,[1] gentle oxidation of dithizone removes two atoms of hydrogen, producing the diphenyl thiocarbodiazone, which is insoluble in alkaline aqueous solution and produces the yellow color noticeable when a solution of partially oxidized dithizone is "stripped" with dilute aqueous ammonia. This intermediate oxidation product can be reverted with such reducing agents as sulfurous acid, thiosulfate, ammonium sulfide, or hydroxylamine hydrochloride (in acid solution). In chloroform solution dithizone oxidation may not be stepwise, and direct oxidation products that cannot be reoxidized may be formed.

Hydroxylamine hydrochloride appears to have little value as a stabilizer for dithizone solutions, but it is effective in the removal of interfering oxidants before the dithizone extraction is applied.[1,2,3] In the mixed-color method for lead the practice of adding 0.1 per cent of hydroxylamine hydrochloride to the standard 1 per cent nitric acid in which the dithizone extract is stripped prior to the color development has been found to yield more stable standard colors.

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[1] *Angew. Chem.*, 50, 919 (1937).
[2] Winkler, *This Journal*, 19, 233 (1936).
[3] Clifford, *Ibid.*, 21, 212 (1938).

SUMMARY

The most stable solutions of dithizone in chloroform were prepared from fresh U.S.P. chloroform further purified by the method of Biddle. Certain precautions to be observed in Biddle's procedure are noted.

The quality of carbon tetrachloride as a dithizone solvent is likewise improved by Biddle's procedure. Storage of carbon tetrachloride solutions of dithizone in the cold and dark under a layer of sulfur dioxide water of about 0.1 *M* strength will preserve them almost indefinitely.

The preparation of stable dithizone solutions in large quantity eliminates the necessity for frequent restandardization.

BOOK REVIEWS

Chemical Analysis of Foods and Food Products. By MORRIS B. JACOBS, 537+xxii pages, D. Van Nostrand Co., Inc., 250 Fourth Avenue, New York, N.Y., 1938. Price $6.00.

The objective of this book as stated in the Preface is "to give systematic coverage to the salient points of the chemical analysis of foods and food products and to include certain of the newer aspects of food analysis such as the chemical assay of vitamins, the detection of the improper pasteurization of milk, the homogenization of milk, the detection of gums and methods for the detection of newer types of sophistication of foods."

Since it is one of the objectives of this book "to give systematic coverage to the salient points of the chemical analysis of foods and food products," one naturally turns for comparison to the accepted standard, *Methods of Analysis of the Association of Official Agricultural Chemists.* The topics covered by Dr. Jacobs' book are in a very large degree those found in *Methods of Analysis, A.O.A.C.*, though some topics are wholly omitted, such, for example, as baking powder and potable waters, while others are rather meagerly treated, such as those of malt beverages and cereals.

Dr. Jacobs has relied in large measure on the A.O.A.C. methods of analysis and has in numerous instances given full credit to this Association. There are, however, numerous instances throughout the book in which this has not been done, but where the wording of the A.O.A.C. methods of analysis has been copied almost verbatim without credit. In some cases the A.O.A.C. wording has been followed, while the only source reference given is that of the original literature. That this procedure is often misleading is quite evident to one familiar with the development of methods by the A.O.A.C., wherein original methods as found in the literature may have undergone more or less marked modifications before adoption. For example, he gives the Fendler-Stüber method for caffeine (page 359), the sole reference in the text being to the original publication in the *Zeitschrift für Untersuchung der Nahrungs und Genussmittel*, yet the details for such method are taken almost verbatim from *Methods of Analysis, A.O.A.C.* A comparison of the Fendler-Stüber method as published in the literature cited and as finally adopted by the A.O.A.C. will show that it has undergone considerable modification. It would have strengthened Dr. Jacobs' book to have given credit in such instances to the A.O.A.C., for the A.O.A.C. methods are essentially official in character and are accepted as the standard methods of analytical procedure.

In the tabular data of the composition of cereals (page 290), no mention is made of two of our most important cereals, namely oats and rice, while buckwheat is included and buckwheat does not properly belong in the class of cereals. Likewise on the same page under the "Composition and Comparison of Flours," but under the general caption of "Cereals," data are given of soybean flour in comparison with cereal flours. This may prove somewhat misleading for the soybean is not a cereal but is a legume.

Dr. Jacobs has included in his book some descriptive matter of various gums, vitamins, sugars, etc., as well as tabular data of the composition of foodstuffs. The chemical structure of some sugars and vitamins are also given. One feature worthy of mention is that several chapters include a discussion of the interpretation of the results of analytical data. These discussions are somewhat brief, and the importance of the subject is such that a marked extension of this feature would have added much to the value of the text. Many of the definitions of the standards under which the Food and Drug Administration operates are also given.

A few errors have been noticed. On page 344, in the description of the method for preparing the phosphotungstic-phosphomolybdic reagent for the determination

of vanillin by the Folin-Denis colorimetric method, directions are given for boiling the solution for 24 hours instead of 1½–2 hours. On page 431, the name of Harrisson is incorrectly spelled as is also the name of Harrel on page 68. On page 447 the expression, "The structural formula for Vitamin A is" has been given twice in succession, one of which should, of course, be deleted. On page 467 the superscript used for reference to the work of Munsell, DeVaney and Kennedy is given as 1 where it should have been 6. On page xxi, paragraph (6), "if" is written for "in."

In general, from the standpoint of one interested in analytical methods, the value of Dr. Jacobs' book lies mainly in bringing together in a readily accessible form some of these methods recently published in the literature.—F. L. DUNLAP.

An Introduction to Microchemical Methods for Senior Students of Chemistry. By CECIL L. WILSON, M.Sc., Ph.D., Senior Assistant in Chemistry, The Queen's University of Belfast. The Chemical Publishing Company of New York, Inc., exclusive agents in North and South America. 1938. viii+196 pp., with 93 diagrams. 19×12.5 cm. Price $3.00.

In the preface of this little book the author states, and quite rightly, that a knowledge of microchemical methods is now essential to every practicing chemist. He also states his belief that this knowledge can be acquired by students in conjunction with their ordinary courses. The text book under consideration is the outcome of this belief and covers the course as taught to senior students by him.

Viewed as a general outline of fundamental operations in microchemistry, to be supplemented by lectures and individual instruction, the book is undoubtedly valuable, but considered as a book of reference or for self study it is rather misleading in the general concept of microchemistry.

There are 187 pages of text, 83 of which in the beginning of the book are devoted to the microscope and its various uses. This subject, microscopy, not microchemistry, deals with the use of the compound and petrographic microscope, classification of crystals, refractive indices, and the preparation of crystals for microscopic examination.

The short chapter that follows treats of spot tests in which the general principles of the subject are developed by examples in connection with tests for a few of the common metals and acid radicals. The next chapter is more extensive and deals with a systematic scheme for qualitative inorganic analysis of the common metals and acids. The micro balance is then discussed and a few examples of inorganic micro-inorganic analysis follow. Volumetric analysis is covered by directions for the estimation of nitrogen and phosphorus.

Two short chapters on the tintometer and the colorimeter then appear, and they are followed by a chapter on organic operations. In this are included examples of crystallization, distillation, filtration, extraction, and sublimation of small quantities of material. Melting points and boiling points are also considered, but very inadequately.

Organic quantitative analysis is treated in less than 2½ pages! Finally, the highly specialized subjects of photomicrography and spectroscopy are treated in the few remaining pages.

The impression obtained throughout the entire work is that each subject is treated inadequately, so much so that it is hard to imagine anyone unfamiliar with the subject obtaining much assistance from it without the loss of time and effort. A few examples in this connection may be cited.

The chapter on the microchemical balance is treated in such a way that, without oral instruction or information from other sources, it would be little short of miraculous if a student could obtain the weight of a single object with a Kuhlmann balance.

In the chapter on organic quantitative analysis, the procedure for the determination of inorganic residues is given as follows: "A few mg. of lead acetate are weighed into a platinum boat, and are then ignited either in the silica combustion tube or in the Pregl-muffle. When the combustion is complete, and the boat has been cooled according to the directions for platinum vessels, it is reweighed."

The procedure for the determination of Kjeldahl nitrogen fails to include the dimensions of the apparatus, or the rate of distillation of the ammonia. The use of copper and perhydrol as catalysts, which is recommended, is poor practice, and the apparatus itself, as shown in the cut, is cumbersome to operate.

A redeeming feature of the book is that at the end of each chapter a bibliography of standard works upon the subjects treated is given. This will greatly aid the student in obtaining the necessary information that the book lacks.—E. P. CLARK.

Outlines of Biochemistry. The Organic Chemistry and the Physicochemical Reactions of Biologically Important Compounds and Systems. By ROSS AIKEN GORTNER. 1017 pp., 85 tables, 165 figures. John Wiley & Sons, New York, 1938. Price $6.00.

While constructed along essentially the same lines as the first edition, this volume has been practically rewritten and includes many of the very latest advances in our knowledge of biochemical problems. In addition, three new chapters have been incorporated, which deal respectively with oxidation-reduction, the flavines, and the hormones.

The text is divided into 7 subsections dealing respectively with (1) the colloidal state of matter; (2) the proteins; (3) the carbohydrates; (4) lignins and the tannins; (5) plant and animal pigments; (6) the lipids and essential oils; and (7) the biocatalysts. These subsections are almost as distinct and separate as individual books; little attempt has been made to effect continuity or establish correlations between them. As might perhaps be expected from the subtitle of the volume, the section on the colloidal state of matter occupies about one-third of the entire text. This is justified by the author in the preface, in which he states that "All of the reactions and interactions which we call life take place in a colloid system—much of the 'vital energy' can in the last analysis be traced back to energies characteristic of surface films and interfaces."

The treatment of the material is eminently factual and practically non-interpretive. It is amply but not exhaustively supported by convenient bibliographic footnotes at the bottom of the text pages.

The volume is replete with instances of practical applications of theoretical principles and it becomes very apparent that the author's interests lean strongly toward the agricultural aspects of biochemistry. While to some the continual reference to practical applications from theoretical science may prove objectionable, it seems to the reviewer that this adds much to the readability of the text, and serves to hold the interest of the student.

The book has been prepared primarily to assist graduate students interested in biological problems, but it must be pointed out that it has a distinctly non-clinical "slant" and hence is not well suited for medical teaching.—HERBERT O. CALVERY.

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